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THE TEMPERATURE EFFECT ON POLLEN FORMATION AND MEIOSIS IN HIERACIUM ROBUSTUM

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THE intricate balance system of meiosis, shown to exist in sexual and apomictic species of *Hieracium* (GENTCHEFF and GUSTAFSSON, 1940; GENTCHEFF, 1941; GUSTAFSSON, 1942), involves four different phenomena: time of prophase start, growth of pollen mother-cells and nuclei, tapetum development, age and situation of stamens and flowers. In the case of normal meiosis these phenomena are precisely and specifically adjusted to one another. In apomicts their mutual relationship is upset in various ways and, as a consequence, meiosis breaks down.

Studies were begun in 1942 for the purpose of working out the conditions of this balance system experimentally. Clone individuals of *Hieracium robustum* were treated with different temperatures. (1) Clone plants were grown in Lund Botanical Garden under natural conditions. (2) Plants were potted on June 1st in a tropical green-house of Lund Botanical Garden and kept under a fairly uniform temperature of 24—26° (centigrades) and a relative air moisture of 85—90 %. (3) Plants were kept for six days under a constant temperature of 5—6° (Svalöf), in sand-mixed soil (*a*) and in mould soil (*b*). (4) Plants were cultivated from June 1st in a dry and warm green-house (Svalöf), likewise in different types of soil (*a* and *b*). Unfortunately, the temperature was not exactly measured in this case. It can be safely assumed, however, that the average maximum and minimum temperatures lay considerably higher in plant series 4 than in series 1 (and 3). Of special value are of course the fixation series 1, 2 and 3, but also series 4 has furnished some data of importance. The date of fixation was in series 1: July 16th, in series 2: July 16th, in series 3: July 9th (*a* and *b*), in series 4: July 9th (*a* and *b*). Most buds occurring were fixed and so a number of different developmental stages were available.

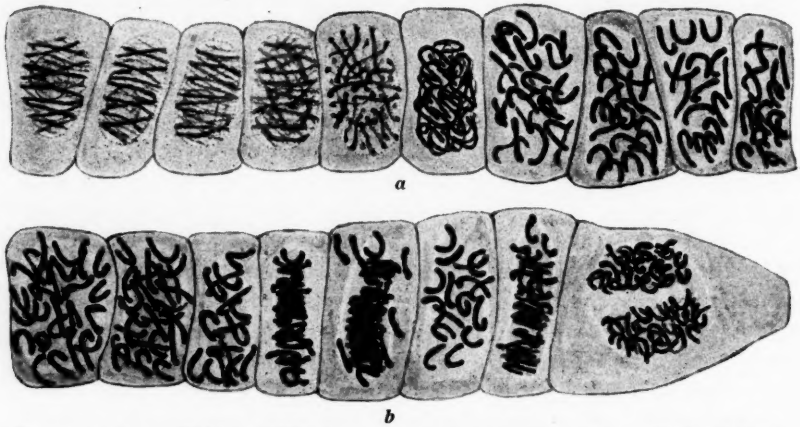
This paper was completed after a lecture on »Embryo sac formation at low temperatures», held by Professor O. ROSENBERG in

the Mendelian Society of Lund. We are indebted to him for encouragement and suggestions.

POLLEN FORMATION.

In their paper of 1940, GENTCHEFF and GUSTAFSSON stated that pollen does not occur in *Hieracium robustum* (p. 213: »never producing pollen»). This finding was confirmed by inspection of the fixed garden material from 1942 (series 1). In no single flower and in no loculus did pollen occur. This was also the case in series 3 (5—6°).

The opposite state of things prevailed, however, in the two greenhouse sets of fixations (series 2 and 4, in *a* as well as in *b*). In series 2



Figs. 1 *a* and *b*. Eighteen PMCs from one and the same loculus. Mitotic division type from prophase to anaphase (5—6°: sand-mixed soil).

every old loculus contained pollen. In earlier stages dyads, tetrads and polyads showed up, the dyads in many loculi being formed with great promptness. The pollen mother-cells never degenerated. In series 4 pollen was most frequently formed, the loculi containing dyads, tetrads and polyads, although the dyad formation was never so regular as in series 2. In addition, however, some loculi showed degenerating pollen mother-cells, and dyads or tetrads could not be traced.

Consequently, in this apomict there occurs an obvious dependence of pollen formation on temperature. At low temperatures no pollen will arise. The higher the temperature, the more regular is pollen formation.

The meiotic conditions underlying these results will be indicated

below. Here it will suffice to point out that chromosome univalence does *not* inhibit pollen formation, since univalents also appear as a rule in series 2, where pollen is always found. Instead, there is an actual *degeneration* of mother-cells at »first» telophase and afterwards in series 1, 3 and infrequently 4. This degeneration is no doubt caused by the greatly upset tissue balance within the loculus. Tapetal cells are at an extremely early stage of growth and division when the prophase of PMCs sets in, and do not catch up with the proceeding PMC divisions. Consequently, pollen formation (as well as normal meiosis) depends on a strictly adjusted tissue development.

The clone, denoted as *H. robustum* MARTR. (non FR.) and now cultivated at Lund and Svalöf, belongs to no Scandinavian apomict. The microspecies carrying this name was described from southern Europe (Tarn in France). The rather high temperature demanded for pollen production accords with the alleged origin and ecology of the clone.

MEIOTIC BREAK-DOWN IN HIERACIUM ROBUSTUM.

In the course of investigations the following division types have been found:

(1) Prophase is mitotic in appearance; chromosomes are long and slender as in vegetative tissue; at metaphase they orientate to form an equatorial plate; at anaphase the chromosomes will be halved, the two chromatids passing to opposite poles. No second division is found.

(2) Prophase is mitotic; chromosomes are long and slender; no complete metaphase plate is formed, the chromosomes being more or less scattered over the spindle and not regularly halved at anaphase; therefore anaphase separation is chromosomal as well as chromatidal. No second division.

(3) Early prophase looks mitotic but diakinesis chromosomes are greatly contracted; metaphase and anaphase behave as in case 2, but the chromosomes are even more scattered; restitution nuclei are frequently formed, leading to unreduced chromosome numbers in the second division (*H. laevigatum* type; ROSENBERG, 1927).

(4) Meiosis proceeds as in case 3 but bivalents are found in variable number (*H. boreale* type; ROSENBERG). Restitution nuclei are common.

(5) Bivalents appear in high number. They orientate and split as in normal meiosis. Univalents are usually passively included in the first-telophase nuclei but they may also split at first anaphase. Restitution nuclei are rare.

(6) In some loculi the first metaphase cells contain twice the somatic number, the prophase chromosomes lying in pairs. Chromosomes are either mitotic (long, slender) or meiotic (contracted, almost spherical) in appearance. Anaphase separation is either chromosomal or chromatidal. No second division occurs. (»Double reproduction»; GENTCHEFF and GUSTAFSSON, l. c.) This division type will be further studied.

(7) The *nuclei* get contracted at prophase stage; the chromosomes regain an interphase appearance; later on metaphase is formed, now with mitotic metaphase chromosomes, and division is mitotic (*H. lacerum*; ROSENBERG).

(8) The chromosomes become highly fragmented, crushed into numerous pieces. No normal anaphase separation or division is possible.

Fig. 2. Mitotic division type (garden material, 1942). In the third cell from the left two metaphase plates are found.

These division types are connected with temperature in a regular manner.

5—6°. — Mitotic divisions (1, transition to 2), doubled chromosome numbers (6). No pollen. Locular contents degenerate.

Garden material. 1939. — Semiheterotypic divisions (3), doubled chromosome numbers (6), bivalent formation (4, 5), nuclear contraction (7), chromosome fragmentation (8). No pollen (?). — *1942*. Mitosis (1, 2), doubled chromosome numbers (6). No pollen. Locular contents degenerate.

24—26°. — Variable bivalent formation (4, infrequently 5). Semiheterotypic divisions (without bivalents) are common, but in these instances pollen mother-cells and tapetal development are of the »normal-meiotical» type, indicating that the bivalent omission depends rather on disturbed chiasma formation than on inhibited chromosome pairing as in mitotic divisions. Restitution nuclei are frequent. Inversion bridges occur sporadically. Some cases of strong fragmentation (8)

were recorded. In 1940 GENTCHEFF and GUSTAFSSON showed that this fragmentation process was restricted to PMCs containing bivalents and that it did not appear until first metaphase or anaphase. In the 1942 material this is also usually the case, but in addition fragmentation takes place at second division. In one pollen-sac a division type exactly similar to that occurring in *H. lacerum* (7) was seen. Pollen is regularly formed. Locular contents were never seen to degenerate.

Warm green-house. — The chromosome behaviour is almost the same as in the preceding instance. In a few loculi degeneration phenomena set in, similar to those in garden material, indicating mitotic-like divisions (1—2). In this degenerating tissue also a few cells show doubled chromosome numbers at prophase and metaphase (6). Some cells with a very high number of bivalents (more than ten) could be observed (5; cf. the 1939 material, GENTCHEFF and GUSTAFSSON, l. c.,

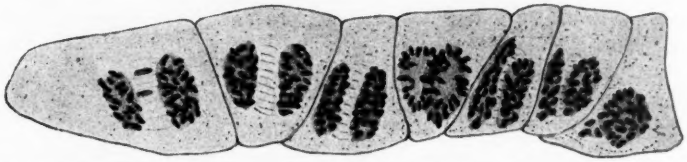


Fig. 3. Seven PMCs in mitotic anaphase-telophase stages. Chromosomes somewhat more contracted than usual. (Garden material, 1942.)

Figs. 19 and 21). Diakineses and metaphases with bivalents are more common than in the 24—26° material. Restitution nuclei and dyads are rather frequent. Prophase nuclei without bivalents have nevertheless the full-grown advanced appearance characteristic of nuclei with bivalents. Inversion bridges have been seen. Pollen is usually formed. Infrequently whole loculi degenerate.

THE BEHAVIOUR OF MITOSIS-LIKE DIVISIONS.

A comparison of prophase and metaphase behaviour in the 1939 and 1942 garden material is very instructive.

In 1939 most semiheterotypic divisions were limited to the central flowers of the head, these flowers being smaller and less developed than the peripheral ones. PMCs were narrow and their nuclei small. The tapetum represented a very young stage of development. The metaphase chromosomes were contracted as in typical meioses (GENTCHEFF and GUSTAFSSON, l. c., Fig. 2). Frequently they filled up the whole cell. Anaphase distribution was irregular, giving one, two or

more daughter-nuclei, and these in most instances degenerated after telophase, since second divisions were never found in these flowers.

In the 1942 material the corresponding divisions behave differently. In most cells the chromosomes are mitotic in shape (Fig. 2). They do not always move regularly on to form an equatorial plate, single chromosomes lying outside the equator and the spindle. Rather frequently the metaphases look entirely mitotic, this being even more so in the 5—6° series. Owing to the small size of the PMCs chromatid separation at anaphase cannot proceed normally. In places, however, where the cells are able to grow larger, for instance, at the top or bottom of a loculus, anaphase and telophase take place as in a normal mitosis. The inhibited chromatid separation at anaphase is consequently, at least partially, caused by lack of space (spindle disturbances?). At late

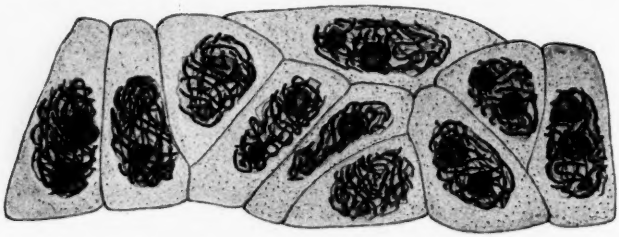


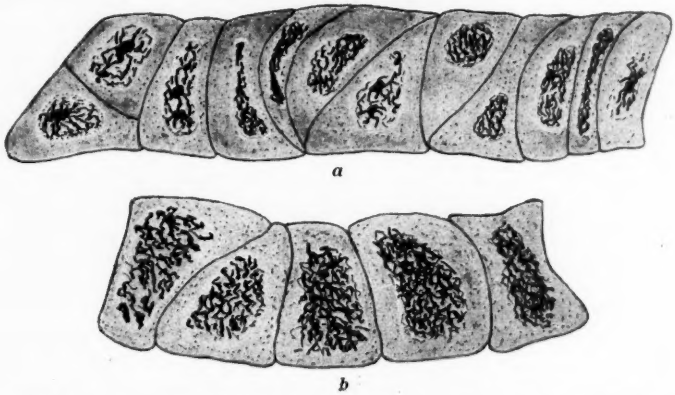
Fig. 4. Mitotic prophase stages (garden material, 1942). Note the irregular appearance of the PMCs.

prophase and early metaphase the cells appear crowded with chromosomes. At late metaphase or anaphase, when the chromosomes are gathered at the equator, nuclear membranes are formed round the chromosomes, giving the new nuclei a flattened shape, and these contain the unreduced number. Probably the telophase membranes form rather early in comparison with the case in common mitosis, so that in some cases the inhibited chromatid separation may depend on this factor also. Sooner or later the cells and nuclei degenerate. No second divisions appear.

In the garden and 5—6° series (1942) the archesporial tissues are often irregularly developed. Most sexual and apomictic *Hieracium* biotypes contain only one row of pollen mother-cells. As shown in Fig. 4, however, the spindles of the last premeiotic mitoses have frequently orientated transversely instead of longitudinally in this material. As a consequence the mother-cells lie displaced in relation to one another. The premeiotic cell-walls are also formed irregularly. This may be

the case with one-rowed PMCs, too, the cells then obtaining a triangular or curved shape. The archesporial disturbances are less frequent in material with bivalent formation, but some small undeveloped cells also may lie beside or even within the row of the full-grown cells. In such a case they contain a degenerated discoloured nucleus and cytoplasm.

In the 1940 paper material was collected regarding cell growth and tapetum development (GENTCHEFF and GUSTAFSSON, l. c., p. 224). It was shown that semiheterotypic divisions occurred in PMCs which were very small and undeveloped and that the tapetal cells contained only



Figs. 5 *a* and *b*. Seventeen PMCs from one loculus. Around the mitotic metaphase plates new nuclear membranes have formed without the chromatids previously separating. Cells and nuclei degenerate. (5—6°: sand-mixed soil.)

one or two nuclei, against four or eight in the case of bivalent formation. Simultaneously flowers showing regularly unpaired chromosomes lay in the middle of the head, representing an early stage of development.

In the 1942 material similar measurements were made (Table 1). Cells with mitotic divisions (fixation series 3 *b*: 5—6°, mould soil) were compared with cells containing condensed univalents (series 4 *b*) and cells with bivalents. Length and breadth of the PMCs were measured. Such a method of measurement is of course rather crude, but, when the differences are considerable as in this case, a more precise but difficult comparison is superfluous.

The data show that the breadth of the PMCs is always approximately identical. The growth and final appearance of PMCs differ entirely,

however, in the two groups of division types. In the case of mitosis and its closest transition stages the PMCs border directly upon each other, their shape being rectangular and their breadth greater than their length. In the case of variable bivalent formation, the PMCs grow considerably at interphase and prophase and finally their length surpasses their breadth. In addition they get free from one another and round off, their shape consequently being ellipsoid rather than rectangular. These differences are very striking. The first-metaphase cells appear to be larger when bivalents occur than in the case of mere singles, but the measurements hardly suffice to bear out this conclusion fully. A similar result was obtained in *Hieracium amplexicaule* (GENTCHEFF and GUSTAFSSON, l. c., Figs. 25—26). Here two distinctly different prophase types occurred. After prophase type 1 semiheterotypic divisions arose (no bivalents, scattered metaphase chromosomes). Prophase type 2 led to pseudohomeotypic divisions (condensed univalents gathering into an equatorial plate) as well as to bivalent formation. Pseudohomeotypic divisions were found in those cells which were on the minus-side of the growth curve. Cells with bivalents lay on the plus-side of the growth curve. Prophase type 1, finally, gave metaphase cells which on the whole were much smaller than those after prophase type 2.

TABLE 1. *The size of PMCs in different types of meiotic break-down in Hieracium robustum.*

	»Mitosis»			»*Hieracium boreale*» type			
	Prophase	Metaphase	Telophase	Prophase	Metaphase		II Telophase
					Univalents	Bivalents	
Length of PMCs..	$M_{10} = 10{,}4\,\mu$	$M_{10} = 11{,}2\,\mu$	$M_{10} = 11{,}7\,\mu$	$M_{38} = 20{,}3\,\mu$	$M_{35} = 20{,}1\,\mu$	$M_{15} = 23{,}9\,\mu$	$M_{10} = 25{,}8\,\mu$
Breadth of PMCs	$M_{10} = 20{,}9\,\mu$	$M_{10} = 20{,}8\,\mu$	$M_{10} = 22{,}2\,\mu$	$M_{38} = 19{,}8\,\mu$	$M_{35} = 19{,}6\,\mu$	$M_{15} = 18{,}2\,\mu$	$M_{10} = 19{,}4\,\mu$

It is obvious that the low temperature of 5—6° for almost one week previous to fixation induced the prophase to start prematurely (precociously) in relation to cell and nucleus growth, as well as to tapetum, flower and head development. But what is the cause of the different behaviour in the garden material of 1939 and 1942? Certainly, in both

years pollen did not form or at any rate was not detected, but also in the most premature divisions of 1939 (central flowers) chromosomes looked meiotic and were distributed irregularly to the two daughter-nuclei (reduction in chromosome number). In 1942 chromosomes were mitotic-like in shape and they gathered more or less regularly to form a mitotic metaphase plate. There are of course several interpretations possible. One of them, the effect of temperature, is particularly probable in the light of the 5—6°-behaviour. In 1942 summer temperature was on an average considerably lower than in 1939, especially during the week when fixations were made (July 13th—20th). The data are shown below:

1942.	June 16 — 23	— 30	— July 6	— 13	— 20
Average maximum temperature	16,9°	19,5°	21,8°	20,9°	19,9° [1]
» minimum »	8,2°	9,9°	11,5°	12,6°	12,4°
Precipitation in mm.	38,6	49,4	—	4,4	9,8
1939.					
Average maximum temperature	24,6°	20,7°	22,5°	20,4°	23,8° [2]
» minimum »	14,0°	11,5°	13,2°	12,5°	13,9°
Precipitation in mm.	11,6	6,7	7,5	27,7	10,0

As shown on p. 2, *H. robustum* reacts to high temperatures by producing pollen. This in turn is conditioned by a precisely adjusted tissue balance within the loculus. At low temperatures pollen formation is inhibited. Similarly, meiosis passes over to mitosis, which in extreme cases is quite typical. Under garden conditions, too, meiotic break-down vill change in different ways, owing to the state of the weather. This difference in behaviour lies entirely in line with the southern origin of the clone denoted as »*H. robustum*».

DISCUSSION AND CONCLUSIONS.

In several agamospermous genera meiosis gets replaced by mitosis also *under normal conditions.* No other instances are known where this happens. Therefore the apomictic phenomena are of prime importance for our understanding of the essentials of meiosis. The classic studies of ROSENBERG (1917, 1927) revealed a series of transitional stages between meiosis and mitosis, but the complete mitosis was not found. The first paper, where this extreme change was entirely analysed

[1] Chromosomes mitotic in shape. [2] Chromosomes meiotic in shape.

morphologically, concerned the *female* development in *Antennaria* (STEBBINS, 1932). Here it was shown that some ovules may undergo meiosis, others pass through mitosis. Since then, this female substitution of meiosis has been found in a great many genera and has been discussed almost to satiety. Prophase is always strikingly delayed. Therefore some authors (BERGMAN, 1935, etc.) regard this division type as a good support of DARLINGTON's precocity theory. However, in addition to this delay of division there occur other features, some of which have been pointed out by GUSTAFSSON, for instance, the vacuolization and growth of the cell during the protracted interphase. He regarded them as the underlying cause. Surveying all the facts, we must admit that the »vacuolization-mitosis» alone cannot at present decide for or against the precocity-theory.

This is definitely the case, however, when the *male* meiosis of *Hieracium* is considered. In its light the female mitosis as well will receive its explanation.

In 1935 GUSTAFSSON supplied some data on male meiosis being replaced by mitosis in *H. lapponicum*, but its lucid properties were not understood. The meiotic balance system, with growth, time and tissue relationships co-ordinated in a distinct manner (GENTCHEFF and GUSTAFSSON, l. c.), showed that the earlier (i. e. the more precocious) the prophase was, the more meiosis broke down. GENTCHEFF (1941) supplied further data on mitosis in *Hieracium umbellatum* (f. apomicta), confirming the idea of a distinct balance system.

In this paper, finally, it has been shown that at low temperatures male meiosis in *H. robustum* is replaced by mitosis, the prophase always being »precocious» in relation to cell and nucleus growth, tapetum and flower development. The same change also appeared under garden conditions in a year when the summer temperature was rather low (1942).

In his paper of 1944, OKSALA has misunderstood the data obtained in *Hieracium*. He says (p. 8): »Wenn nun in einer Pollenmutterzelle die Frühreife der Prophase, *nach dem Entwicklungsstadium des Tapetums beurteilt* (italics by the present authors), vorliegt, so braucht dies keineswegs notwendigerweise zu bedeuten, dass die Prophase auch hinsichtlich der Entwicklungsstufe der Chromosomen derselben Zelle frühreif wäre und umgekehrt. Die von GUSTAFSSON gemeinte Frühreife der Prophase oder ihr Fehlen hat also, wie mir scheint, nichts mit DARLINGTONS Präkozität zu tun, die *ein bestimmtes Zeitverhältnis der chromosomalen und extrachromosomalen Entwicklungszyklen der gleichen*

Zelle darstellt» (italics partially by us). This italicized clause describes excellently, however, what has been found in *Hieracium*. The italicized sentence given first rests upon a misconstruction. OKSALA's results on premeiotic mitoses in *Aeschna* are valuable from a morphological point of view but, as far as we can see, they have no bearing whatsoever on the mechanism of meiosis.

In a certain ontogenetic stage of phanerogams the vegetative phase of development is altered in a generative direction, i. e. flowers are formed. This change is thought to be produced by special bloom hormones (MELCHERS, 1939, 1942). Similarly, at a dinstinct stage of the generative development the archesporial tissue gains new physiological properties, by which meiosis sets to work. Long ago it was claimed that this change was effected by special meiosis-producing substances or hormones. As early as in 1909—1910 FUJII postulated that »meiotic divisions are probably caused by chemical substances» (ISHIKAWA, 1911), and during the last decade this view has been repeatedly projected. Discussions on this point are found in GUSTAFSSON, 1942.

Studies on agamospermous genera have consequently shown that meiosis is replaced by mitosis, either when prophase sets in extremely early (precociously) or when it is extremely delayed. *In both extremes the tissue balance is strikingly upset.* When prophase is curtailed, cells and nuclei do not reach their normal (optimal) size and the tapetum is still undeveloped. When prophase is delayed, cells and nuclei grow considerably, vacuolization of the plasm sets in and the tapetum becomes dissolved. From the hormonal standpoint (which is so far entirely hypothetical) this should imply that meiosis-hormones function only at a distinct moment and at a precise physiological state of the cell. The more the spore mother-cells deviate from their normal condition, the less effective are the hormones. Such transitions from mitosis to full bivalent-meiosis exist in *Hieracium robustum*. Here meiotic and mitotic properties intermingle in various ways. Finally, in some apomicts the formation of the meiosis-producing substances may be inhibited or the amount insufficient for a regular meiosis.

Further experimental work will be carried out in order to elucidate these questions.

In his paper of 1941, BERGMAN showed that agamospermy is not stable in *Hieracium* subg. *Archieracium*. Here, as well as in *Antennaria* (STEBBINS, l. c.), some ovules enter meiosis, others pass through mitosis. The unreduced gametophyte generally originates by the latter process, although other possibilities also exist. Reduced and fertilized egg-cells

have probably played an important rôle for the genesis of this genus, even if they should by now be of lower value for the origin of new biotypes. *Asexual* aberrants may arise in apomicts lacking functioning pollen via special processes in the EMCs which lead to unreduced egg-cells (restitution nuclei, pseudohomeotypic divisions, etc.). Some apomicts show a high percentage of meiotic ovules (*H. scandinaviorum* with 56 % meioses). In other apomicts mitoses predominate (*H. rigidum* with 100 % mitoses).

In view of what has been described for male meiosis in *H. robustum* we may postulate that the female type of division is also influenced by environmental conditions, for instance, temperature. If a full parallelism exists, cold summers will give a surplus of mitoses, hot summers a surplus of meioses. In the last-mentioned instance the formation of new apomicts in nature may be greatly enhanced. We may further conceive that at the outskirts of a distribution area, where an apomict meets with new environmental conditions, it will show an increased ability to produce asexual aberrants.

DAHLSTEDT pointed out in 1907 (a and b) that Scandinavian representatives of the *Taraxacum* group *Palustria* often lack pollen, while their close relatives in Middle- and South-Europe form pollen abundantly. A similar thing has been shown for apomicts in the group *Erythrosperma* (1920). (*T. rubicundum* * *monspeliense*, presumably introduced late to Sweden, produces pollen. Its Scandinavian relative *T. rubicundum* lacks pollen.) One and the same microspecies may contain individuals with and without pollen (*T. laetum* * *obscurans* and its f. *egens*). An apomict, usually giving pollen, will under certain environmental conditions inhibit pollen formation. — In *Archieracium* such phenomena have not been thoroughly studied, but the findings in *H. robustum* indicate that the development or non-development of pollen in one and the same apomict is not due to biotypical heterogeneity but to environmental influences. The remarkable morphological findings in *Taraxacum*, where no other exterior character but the ability to produce pollen may distinguish two forms, suggest that there is also here a sort of climatical control of the meiotic system.

SUMMARY.

(1) This paper records a preliminary series of experiments undertaken in order to find out the mechanism of the meiotic balance system in sexuals and apomicts. The material consisted of clone individuals of *Hieracium robustum* MARTR.

(2) Under garden conditions and at a temperature of 5—6° no pollen is produced. At 24—26° pollen forms abundantly. Plants placed in a hot and dry green-house produce pollen, although it is not found in every loculus owing to a partial degeneration of PMCs. The altered tissue balance within the loculi is held to be responsible for this temperature effect.

(3) Meiosis is changed to mitosis, more or less typical, under the garden conditions of 1942. This change is even more conspicuous in the 5—6° material. In 1939 the garden material showed meiotic chromosomes, usually being unpaired, although bivalents arose in peripheral flowers. The different behaviour of the 1939 and 1942 material is probably due to differences in summer temperature, 1939 having been hotter than 1942.

(4) In material cultivated at high temperature mitotic-like chromosomes never occur. Instead of that, a high number of bivalents may be found.

(5) *The change from meiosis to mitosis is always accompanied by a premature (precocious) onset of prophase,* relative to growth of pollen mother-cells and nuclei, tapetum development, age and size of flowers.

(6) In the light of the findings reported, the mechanism of meiosis, as well as certain apomict problems, are briefly discussed.

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MORPHOLOGY AND CHEMISTRY OF THE METAPHASE SPINDLE

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INTRODUCTION.

ACCORDING to EHRENBERG and ÖSTERGREN (1942), the shape of the spindle is subject to variation at each phase of the cell division. This variation has been made the subject of study in the present paper, interest being centered on the laws which it obeys. An attempt has also been made to throw some light on the forces which take an active part in the process causing this variation. As the spindle is an active factor of major importance in the process of mitosis, its variation in shape has been connected up as far as possible with some measurable property of the mitosis in its entirety, i. e. in the first place with the rate at which the chromosomes move.

The views advanced in this paper are by no means conclusive; much remains to be clarified and experiments to this end are in progress.

According to BARBER (1939), temperature has a strong influence on the speed at which the chromosomes move during anaphase. It is, therefore, important to ascertain whether the spindle shows any temperature-conditioned variation within the range of temperatures at which normal growth occurs. A measurable property of the spindle is the curvature of its outline. [BERNAL and FANKUCHEN (1941) have found the radius of the curvature to be a constant property of the tactoids formed in tobacco mosaic virus solutions.] This curvature is appreciable if a cell at metaphase is examined at right angles to the direction of the nuclear division (Fig. 1 *a*, *b*). Preliminary examinations of root meristems of plants cultivated at different temperatures showed that the curvature varied considerably at one and the same temperature, and repeated measurements had to be made until different statistically significant mean values could be established. The possibility was conceived that numerous factors may have an influence on the property to be measured, i. e. the curvature, and that, therefore, the factor on whose action interest centers in this paper might be overshadowed by them.

In addition to temperature, the following factors may be considered as possible causes of the variation:

(1) A hereditary variation, i. e. a variation between different biotypes.

(2) A variation between roots showing different rates of growth.

(3) A variation between roots showing the same rates of growth, though at different stages of the cycle of periodicity. (Roots, as a rule, grow periodically; see below.)

(4) A variation between different parts of one and the same root.

(5) The difficulty of accurately defining the stage of metaphase.

(6) The variation due to defective measurements (the difficulty of regularly reproducing clearly outlined spindles as well as the investigator's subjective interpretation of the image seen in the microscope).

With a view to neutralizing, as far as possible, the influence of those factors which complicate the analysis, *Salix fragilis* × *alba*, a tree growing in the South of Sweden, was considered suitable for the experiment. From a single *Salix fragilis* × *alba* tree it is possible to obtain an unlimited number of cuttings which, if allowed to remain in water for some time, yield a large number of roots representing a genotypically homogeneous material. The chromosomes of *Salix* are numerous and small, a fact which causes the metaphase plate to have the appearance of a uniformly thick line when viewed from the side, and which makes the precise definition of the entire metaphase possible. Moreover, the chromosome arms which might conceal the structure of the spindle are absent.

Growth periodicity of Salix roots. — In a preliminary experiment the periodicity of the growth rate was studied. From the moment they had been cut from the tree the cuttings were cultivated in darkness in glass jars filled with tap water. (The same method of cultivation was used in the experiments described later in this paper.) At the end of about 12 days roots had developed measuring a few centimetres in length. The lengths of these were measured every second hour during 72 hours. At temperatures ranging between 10° and 30° C, a distinct 8-hours' periodicity was observed, the growth of the roots showing 3 maxima and 3 minima of growth per 24 hours. Statistically viewed this means that there were significant differences in growth rate between intervals of 2 hours ($P < 0{,}001$; see BONNIER and TEDIN, 1940), whereas the variation between intervals of 24 hours and of 8 hours was inconsiderable ($P > 0{,}2$).

METHOD.

Cuttings of *Salix* measuring about 1,5 dm in length were cultivated in accordance with the periodicity experiment. Three days before being fixed the material was moved into thermostatically controlled chambers which, though rather simple appliances, kept the temperature fairly constant at 11,5°, 16,5°, 23,5°, and 29,0° C. The growth speed was determined 24 hours before the roots were fixed. For the purpose of eliminating any possible influence of periodicity when making the calculations, 3 roots were fixed each hour at each temperature during the course of 8 hours. The roots were fixed in chrome-acetic-acid formalin after KARPECHENKO's method, this process bringing out the

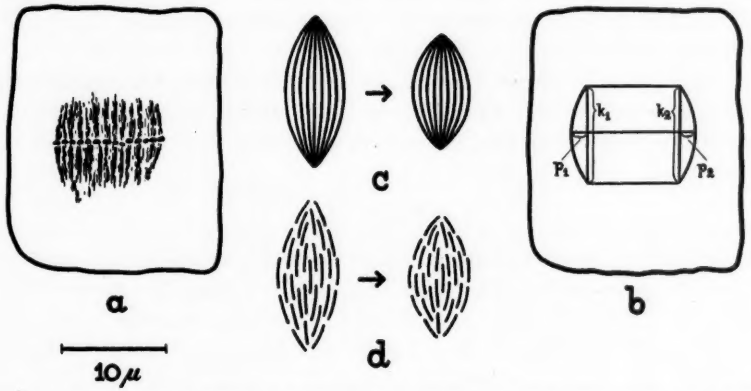

Fig. 1. *a*: the mitotic figure of *Salix*; *b*: the spindle schematically drawn; *c* and *d*: the curving of the outline of biacuminate spindles, caused by the contraction of their micelles.

spindle in full relief, while the sections were stained in iron-aceto-carmine for about 12 hours. Longitudinal sections of the roots were made measuring 12 μ in thickness. EHRENBERG and ÖSTERGREN (1942) used the same method. With the help of a drawing-apparatus drawings were made of five spindles selected at random from each root. In order to prevent the worker from following his own suggestions when selecting the spindles, the preparations were mixed before the drawings were made and re-numbered by another person.

The sections indicated by k and p (Fig. 1) were measured. Owing to the available instruments being rather defective, it is sometimes impossible to differentiate the outline of the spindle from a straight line. In such cases the value of the radius of curvature $=\infty$, a value that

cannot be used for further calculations. It is, therefore, more adequate to base the calculations on the curvature which represents the inverted value of the radius and which in such cases is equivalent to a value of 0. According to the Pythagorean principle, the curvature is $\frac{8p}{4p^2 + k^2}$ units of length^{-1}, if one sets out from the assumption that the outline of the spindle is a part of the periphery of a circle. Thus, this value approximately expresses the mean curvature. In the following calculations 1 unit of length is equivalent to $^2/_3\ \mu$ and 1 unit of curvature to $\frac{3}{2000}\ \mu^{-1}$. The measurements were made with an accuracy of 0,1 unit of length. An error of 0,1 unit of length gave a maximum error of about 1 unit of curvature. The curvature was, therefore, calculated in whole numbers only.

Fig. 1 shows that two measurements of the curvature of each spindle were made, a fact that permitted the investigation of the variation between the cells within one and the same root.

RESULTS.

Variation in temperature. — The statistical treatment of the material consisted in hierarchically classifying it according to the methods described by BONNIER and TEDIN (1940). The only external factor that was modified was temperature. Temperature was not the solitary factor

TABLE 1. *Analysis of variance. Influence of temperature and fixation time on the curvature of the outline of the spindle.*

	Degrees of freedom	Sum of squares	Mean square	Row No.	Quotient of variance
Between temperatures ...	3	99079,27	33026,423	I	$\frac{\text{I}}{\text{III}} = 25{,}93$
Within temperatures.....	906	289978,28	320,064	II	
Beetwen times of fixation	28	35651,34	1273,262	III	$\frac{\text{III}}{\text{V}} = 1{,}76$
Within times of fixation	878	254326,94	289,666	IV	
Between roots	59	42732,74	724,284	V	$\frac{\text{V}}{\text{VII}} = 2{,}02$
Within roots	819	211594,20	258,357	VI	
Between spindles	364	130423,20	358,305	VII	$\frac{\text{VII}}{\text{VIII}} = 2{,}01$
Within spindles (Error)...	455	81171,00	178,398	VIII	
Total	909	389057,55			

inducing the gross variation in the radius of the curvature of the spindle, a fact that may be read off from Table 1 illustrating the analysis and showing the distribution of this variation. Table 1 furnishes the following information:

(1) The quotient of variance between the mean squares in the rows VII and VIII in the table [here expressed by $v^2\left(\frac{\text{VII}}{\text{VIII}}\right)$] indicates with great certainty that the variation in curvature of the spindle within one and the same root and which is appreciable when comparing the cells with one another is not merely an accidental one. If the probability were 0,001 or $P = 0{,}001$ (cf. BONNIER and TEDIN, 1940) that only accidental causative agents are at work, the quotient would have had to be 1,36 (calculated from BONNIER and TEDIN's formula on p. 321).

(2) Evidence having been furnished that the subordinate classification indicated in the rows VII and VIII in the table was established on reliable grounds, the question whether true or only accidental differences exist between the roots can only be clarified by comparing $v^2\left(\frac{\text{V}}{\text{VII}}\right)$ with the required v^2 value for $P = 0{,}001$ calculated from the above-mentioned formula. Thus the quotient 2,02 (for $P = 0{,}001$ calculated to be 1,75) gives rise to the belief that differences really do exist. The question of whether these differences really differentiate the roots from one another or whether they are caused by the fact that the fixing of the roots had probably been made at different stages of the cycle of growth periodicity cannot be cleared up on the basis of this analysis.

(3) $v^2\left(\frac{\text{III}}{\text{V}}\right)$ doubtless hardly indicates any probability at all ($P = 0{,}05$) that the comparison of the different points of time at which the roots were fixed, and on which the first subordinate classification was based, is justifiable. There is, however, a probability that the radius of curvature varies periodically, and it is possible that the high value of $v^2\left(\frac{\text{V}}{\text{VII}}\right)$ was partly due to the fact that the cuttings showed individual periodicity. Having grown in the dark for about two weeks, they might have fallen out of the rhythm of periodicity which they originally had in common.

(4) The reliability of the superordinal classification in »between» and »within» the temperatures may be conclusively assessed by determining different values of v^2. As, however, the classification in the

rows III and IV is not entirely without significance, and to safeguard overestimating the probability that temperature is responsible for the variation, $v^2 \left(\frac{I}{III}\right)$ should be determined. But even this quotient, which statistically is very unfavourable, expresses a high degree of probability.

Summarizing the data obtained by this analysis it may be said that

(1) temperature doubtless has an influence on the degree of the curvature of the spindle;

(2) significant differences exist between the roots at one and the same temperature, and that these differences are in all probability dependent partly on periodicity;

(3) significant differences are disclosed on comparing the cells of one and the same root.

TABLE 2. *Curvature of the spindle and growth speed of the roots.*

Temperature °C.	Number of measurements (= 2 × No. of spindles)	Mean curvature, units (see text)	Mean error	Root growth per 24 hours mm	Mean error
3	10	81,50	5,89	ca. 0,1	
11,5	230	65,57	1,13	1,78	0,12
16,5	220	61,57	1,07	4,74	0,34
23,5	230	44,52	1,35	9,56	0,68
29,0	240	41,52	1,16	14,13	1,17

Table 2 shows that *a fall in temperature is expressed by an increase in curvature of the outline of the spindle.* The table also includes a value for 3° C. As a rule, cell division does not take place at such a low temperature, but in 1 out of 10 of the roots investigated at this temperature a few mitoses nevertheless occurred, and on these the measurements were based.

Variation within and between the roots. — The chief purpose of the present investigation was to shed some light on the problem of whether any correlation existed between the variation in the shape of the spindle and the speed at which the chromosomes moved. To calculate, with any degree of accuracy, the speed at which the different phases of mitosis are traversed would require a comprehensive material of fixed preparations (cf. TISCHLER, 1942). It may, however, be stated that the growth rate of the roots per 24 hours (Table 2), the speed of the chromosome movements at anaphase (BARBER) as well as the radius of curvature of the spindle (= inverted value of the curvature) are

positively correlated to temperature. To what extent this correlation is functionally direct is a question that further experiments will have to clarify. In the present material the curvature varied grossly in the roots at one and the same temperature. The causes of this variation are worthy of discussion. In the material reported in this paper a variation in the growth speed, though fairly inconsiderable because of the fact that the roots resembled one another very much, was observed. So far, the view is justified that the accelerated growth speed at one and the same temperature is due not only to an increase in the frequency of division, but also to an accelerated speed of mitosis. (If the variation in temperature is also included, this view conforms with BARBER's observations.) If this is true, a connection between the shape of the spindle and the speed of the movements of the chromosomes would be

TABLE 3. *Curvature of the spindles in different root parts at 29,0° C (3 roots) and 11,5° C (1 root).*

Temperature °C.	Root No.	Curvature of the spindle (in brackets number of measurements)			
		Plerome	Periblem	Mean value of the root	Calyptrogen
29,0	322	(16) 66,25	(28) 30,68	43,64	
	352	(24) 55,63	(26) 31,73	43,20	
	413	(28) 59,54	(18) 26,67	46,67	
	Total	(68) 59,74	(72) 30,06		(6) 65,57
11,5	472	(14) 72,93	(18) 55,78	63,28	

expressed by a regression between the curvature and the growth speed. A covariance-analysis along the lines described by BONNIER and TEDIN within the two highest temperatures at which the individual root measurements yielded fairly significant values showed that there was a negative regression with a probability ranging between 0,80 and 0,95 ($0{,}2 > P > 0{,}05$). This result is merely suggestive and in itself does not furnish any explanation of the gross variation between the roots.

The experiments disclosed further that the outline of the spindle was, as a rule, more curved in the cells of the central parts of the roots. A statistical analysis showed that the variation within the roots was to a very large extent due to the variation between the plerome and the periblem, the spindles of the periblem cells being less curved (Table 3). There occurs a further though less marked variation within the plerome and periblem respectively. This variation may be due to the fact that

the metaphases were of different ages. Table 3 also contains the mean value of some measurements made in the calyptrogen where the curvature is most strongly pronounced.

Mitoses within one root are apt to occur chiefly within the periblem or the plerome, a fact that is rather striking to notice. It is most likely due to a pulsating periodicity during which these parts of the root are alternately in lively mitosis activity. The result is that the gross variation between the roots $\left[v^2\left(\frac{V}{VII}\right)\right.$; see Table 1$\left.\right]$ is, to a large extent, due to the fact that the mean values of the plerome and periblem are alternately predominant. Possibly, the variation in »between the times» $\left[v^2\left(\frac{III}{V}\right)\right.$; see Table 1$\left.\right]$ is connected with this pulsating periodicity. In the earlier measurements (Tables 1 and 2) the present writer has not been able to avoid some selection towards spindles with small curvature at high temperatures. Periblem cells predominate in the 29° drawings owing to the readiness of the plasma to stain (even on weak staining of the periblem the colouring matter is apt to act too powerfully on the plerome cells so that the structure of their spindles cannot be estimated). At low temperatures this difference in the plasmas is less marked. This defectiveness of the measurements makes the differences obtained between temperatures (Table 2) somewhat too great. However, the differences in curvature between temperatures within plerome and within periblem (Table 3) are statistically significant.

Physiological differences between the plerome and the periblem. — The cells of the plerome are frequently so narrow that the chromosome plate at metaphase is forced, for purely mechanical reasons, to take an oblique position, a factor which most likely plays an important part. It appears that the spindles which, for some reason or other, orientate themselves lengthwise in a direction deviating from the growth direction show, as a rule, a greater curvature. Generally speaking, this fact might be interpreted as a divergency from the mechanism which controls the polarity of the root. Similar reasons might account for the great degree of curvature in the calyptrogen.

The hydrogen-ion activity of the plasma is another factor that most likely influences most phenomena of life. STRUGGER (cit. by TISCHLER, 1942, p. 11) demonstrated that the value of the pH in meristem cells was equal to 3,47, in the stretching zone to 6,84, and in the differentiating zone to 7,62. It is not unlikely that there is some relation between the increased hydrogen activity in the meristem and the readiness to divide.

For the purpose of demonstrating to what extent pH differences exist between the plerome and the periblem the »range indicator method» described by SMALL (1929) was used. All precautionary measures advised by SMALL (1929, p. 59) having been strictly followed, the results obtained cannot but suggest the existence of true pH differences. As regards the plerome it was definitely observed that the pH value lies somewhere between 5,6 and 5,9 (see Table 4). In the periblem the three or four outer cell-layers differ from the other ones chiefly by reason of the fact that they show greater readiness to stain. The pH value of these outer cells is most likely 4,4, whereas in the remaining periblem the pH value lies between 4,8 and 5,2. The change of colour to red which occurs in benzene-azo-α-naphthylamine after two hours' treatment, is most likely due to the latter's toxicity (cf. SMALL, l. c., p. 64). Table 4 shows that there is a gross variation in the reaction of the periblem cells to methyl red. Red, however, is the predominating colour, though the orange staining occurring in some cells may be due to a tinge of alkaline colouring. The definite low pH value of the outer periblem is in agreement with the measurements made by MARTIN on *Helianthus* (cf. SMALL, l. c., p. 185). On the other hand, MARTIN found the same pH in both the periblem and the plerome.

In brief, the results of the pH determinations were as follows:

Plerome pH = 5,6—5,9

Periblem { inner pH = 4,8—5,2
outer pH = 4,4

The effect of colchicine-mitotic substances on the shape of the spindle. — In this connection some observations on the shape of the spindle will be briefly reported. These were made after treatment of the roots with C-mitotic substances, i. e. with substances which, like colchicine, have an inactivating and dissolving effect on the spindle. An attempt was made to determine the threshold value of the effectiveness of some substances. The conditions under which the experiments were made were identical with those described by LEVAN and ÖSTERGREN (1943), and a series of solutions in predetermined concentrations was used.

Of the preparations used for these experiments it was observed that the roots treated with

(1) somewhat weaker concentrations than those which may induce C-mitosis,

TABLE 4. *Colour of the different root-tissues in indicator solutions.*

Indicator	BTB	BCP	MR	BAN	BCG and BPB
Number of examined sections	5	3	11	8	1 in each
Conditioning effect of the indicator in minutes	30—120	5—30	15—120	10—120	BCG: 30 BPB: 5
Colour of the plerome	yellow	yellow	light yellow-yellow	yellow	green green-blue respectively
pH of the plerome	$< 6{,}2$	$< 5{,}9$	$> 5{,}6$	$> 4{,}8$	$> 4{,}4$
Colour of the 3—4 outer layers of the periblem	yellow	yellow	red	as a rule red (exceptionally yellow after 120 min.)	green (-blue)
Colour of inner periblem	yellow	yellow	5 sect. (20—120 min.) red 2 sect. (15—20 min.) pink 2 sect. (25—120 min.) red-orange-yellow cells 2 sect. (80—90 min.) no staining, a few scattered red cells	4 sect. (10—30 min.) yellow 4 sect. (120 min.) red	green (-blue)
pH of the periblem	$< 6{,}2$	$< 5{,}9$	$< 5{,}2$	outer: $\leq 4{,}4$ inner probably: $> 4{,}8$	$> 4{,}4$

BTB = bromthymol blue, BCP = bromcresol purple, MR = methyl red, BAN = benzene-azo-α-naphthylamine, BCG = bromcresol green, BPB = bromphenol blue.

(2) with concentrations that may induce C-mitosis at a point preceding that at which the spindle is completely dissolved,
showed a greater curvature of the spindles than the control roots treated with pure water.

Thus, the value of the curvature after 4 hours' treatment with 2,0-C ethyl alcohol was equal to 76,68 units. After an equally long treatment with 0,0625-C ethyl alcohol, the value of the curvature was only equal to 46,80 units. In this case the measurements were taken in the periblem only. The difference between these values is statistically significant. The stronger concentration induced C-mitosis in 12 hours and killed the root. The weaker concentration did not have any appreciable effect on cell division. As regards colchicine the conditions are analogous, and pure hydro-carbons, too, show a tendency in this direction.

It should be pointed out that the material experimented upon was too limited to permit of the elucidation of the entire process of dissolution of the spindle. Other facts which were disclosed in the course of the experiments are outside the scope of the present report. Specific effects or a prolonged action of the substance in question account for them.

DISCUSSION.

The variation in the shape of the spindle depends on the variation in the shape of the protein molecules. — After fixation the microscopic picture of the spindle shows a bundle of fibres, the longitudinal direction of which coincides with the direction of division. The question whether these fibres are artefacts has been much discussed. At all events they show that the majority of the small parts of the spindle are orientated in this direction. The fact that SCHMIDT (*e. g.* 1939) *et al.* found that the substance of the spindle was positively birefringent furnished further evidence that the spindle consists of symmetrically arranged fibrils. These are most likely made up of polypeptide chains (cf. FREY-WYSSLING, 1938).

From the preceding statements it may be inferred that an increase in the curvature of the outline of the spindle is in all probability correlated to the following defined changes of environment:

(1) Fall of temperature.

(2) Rise of the pH, i. e. in the plerome as compared with the periblem, the pH of which is lower.

(3) Addition of organic substances.

Determination of the appearance of the living spindle of *Salix* encounters great difficulty. Only fixed and stained cells were investigated. Owing to the violence of the method of preparation the experimental material is doubtless altered, at any rate to a certain extent. As, however, the same method of preparation was applied throughout the whole investigation the observed regularity with which the variation occurs is bound to correspond with some analogous variation in the living cells.

The morphological variation in the spindle which is perceptible in the microscope is no doubt connected with variations in its compounds, i. e. with variations in the protein molecules. The literature is rich in publications dealing with the contractibility of the polypeptide chains (see FREY-WYSSLING's survey). Among these, ASTBURY's investigations of roentgenograms of drawn-out and contracted hair (1933) are illustrative. This author demonstrated that folded polypeptide chains (α-keratin) could be transformed into drawn-out ones (β-keratin). Most likely similar intramolecular reactions play a part in the contraction of muscles. SERRA (1942) is of the conjectural opinion that the spindle is formed during prophase by a stretching-out of formerly scrunched-up protein molecules. According to ÖSTERGREN (1944), it may be possible that the colchicine effect induces a contraction of the polypeptide chains to a shape which prevents the chains from arranging themselves parallel to one another in a spindle.

It is possible that the variation in the shape of the metaphase spindle is due to processes of contraction and drawing-out of the protein molecules. A drawing-out of the molecules would induce a straightening of the outline of the spindle. It is very difficult to furnish mechanical evidence of the existence of such a correlation, though many more or less direct reasons support this view, as for instance:

(1) In a biacuminate spindle a contraction (and thickening) of the molecules composing it causes a greater curvature of the outline. This process takes place irrespective of whether the fibrils (molecules) are of the same length as the spindle (Fig. 1 *c*) or shorter (Fig. 1 *d*). It is essential, however, that the poles of the spindle as regards their position are not fixed in the plasma, a fact which would most likely cause the reverse to happen. Electrophoresis tested on spindles of *Tradescantia* (KAMIYA, 1937) revealed that the poles of the spindle were not fixed, as the whole spindle could move about within the cell. The same effect may be brought about in the spindle of *Salix*, which is usually barrel-shaped, if the two extreme flat surfaces of the barrel are composed of

lamellae of constant size. When the method described in this paper was used, however, the top and bottom contours of the spindle were always blurred, a fact which made the determination of their size impossible. With the contraction of the molecules and the curving of the outline the absolute height of the spindle should decrease too (cf. Fig. 1 *c*, *d*). In good agreement with this assumption is the observation made in the course of the temperature experiments, namely that the spindles of greatest absolute length occurred at the highest temperature at which the outline was simultaneously straightest.

(2) The contraction of the molecules depends on the formation of chemical links between the small parts of the molecules. Simultaneously with the formation of these links similar bonds may occur between the molecules. Owing to the fact that under normal conditions the chromosomes at metaphase seem to repel each other (irrespective of whether this occurs electrostatistically or mechanically) and thus draw out the equatorial part of the spindle, the formation of the intermolecular bonds mentioned causes the tying-up of the spindle at the poles. Thus the primary cause underlying the contraction of the molecules also accounts for the curving of the outline.

(3) The assumption that the curving of the outline of the spindle is a stage in the metamorphosis toward the inactive non-existing (?) spindle of C-mitosis supports this hypothesis. When the contraction and thickening of the protein molecules have attained a certain degree, the surface with which they can possibly attract one another is so small that dissolution of the spindle may occur (cf. ÖSTERGREN, 1944).

(4) All chemical linkages likely to take an active part in the process of contraction have such thermodynamic properties that a fall of temperature directly causes a contraction of the molecules (cf. below).

(5) Simultaneously with the curving of the outline of the spindle a contraction of the chromosomes in the same cell occurs. The chromosomes as well as the spindle are fibrillar protein structures. According to ÖSTERGREN (1944), C-mitotic substances have this effect on the chromosomes. In addition, it is also known that refrigeration of the plants for the purpose of fixation induces a contraction of the chromosomes. The question whether the chromosomes are shorter in the plerome than in the periblem has not yet been investigated.

Until the reverse is proved it is therefore justifiable to interprete the perceptible, statistically demonstrable variation in the curvature of the outline of the spindle as a function of a variation in the shape of the protein molecules which are not perceptible under the microscope.

In this connection, however, it should be pointed out that the breaking-up of the molecules might bring about a similar effect. SVEDBERG (1937) observed that pH alterations reversibly have such an effect. The undivided molecules may undoubtedly be subject to variation in shape. Therefore it is reasonable to assume that if the breaking-up of the molecules is one of the factors inducing the variation, this process is of minor importance — at least until more light is shed on the conditions on which this breaking-up depends. The shape of the spindle will probably depend on the hydratation condition too, but this will not be discussed in this paper.

The chemical forces at work in the contraction of the polypeptide chains. — It is clear that linkages between the groups of atoms of the main chain as well as between those of side chains induce a contraction of the protein molecules. In the following the chemical linkages which are apt to occur will be recalled in brief.

(1) VAN DER WAAL's forces between the lipophil groups. The power of attraction which is active between lipophil groups needs extremely little additional energy to be neutralized. The lipophil affinity existing between amino-acids situated close to one another may cause the contraction of molecules, especially if the acid residues are terminated by phenyl and other hydrocarbon radicals. On account of its weakness this linkage is to a high degree dependent on temperature, a fact which agrees with the reported results. The linkage does not show any direct dependence on the pH. ÖSTERGREN (1944) supports the view that the mutual attraction between the lipophil groups plays an important part in the contraction of the polypeptide chains.

(2) The attraction between groups having a marked dipole moment might also bring about the contraction of molecules. Several amino-acids contain such atom configurations, for instance the OH- and NH_2-groups. With this kind of bond the degree of hydratation plays an important rôle (cf. FREY-WYSSLING, l. c., p. 221) which, in its turn, depends not only on the concentration of neutral salts, but also on the pH.

(3) Ionic linkages. Provided acid and basic amino-acid residues are arranged in such a manner that their position to one another is a favourable one, they may, simultaneously with their dissociation, partake in an intrinsic salt formation. In other words, the amino-groups present in the shape of ammonium groups ($—NH_3^+$) and the carboxyl groups present in the shape of carboxylic-acid anions ($—COO^-$) attract one another with pure electrostatic forces. Such ionic bonds are largest

in number at the iso-electric point, and there is a possibility of a relation existing between them and the observation that the pH of cells showing a readiness to divide is far below the I.E.P. (see TISCHLER, l. c., p. 12 *et seq.*). PFEIFFER assessed the I.E.P. of the protoplasm in the tips of the roots of *Hyacinthus* and of some Leguminous plants at pH 4,3 (FREY-WYSSLING, l. c., p. 127), and, as a rule, its value is far below the neutral point pH 7. The protoplasm of *Salix* roots most likely also has an »acid» I.E.P., and the pH value of the plerome established to be 5,6—5,9 (see Table 4) would indicate a shift from the I.E.P. as compared with that of the periblem. As, however, simultaneously the protein molecules of the spindle contract, it is not likely that the ionic linkages have a conclusive bearing on the molecules. This possibility, however, should not be overlooked as long as the I.E.P. of the spindle is still obscure. Besides, it is not impossible that under certain circumstances the effect of a reaction in one direction may be overshadowed by an antagonistic and more powerful effect of an other reaction.

(4) Covalent bonds. In amino-acids which make up polypeptide chains there are many possibilities of acid amide-, ether-, and other bonds arising. Owing to the great activation energy the formation and dissociation of such bonds — except when special enzymes are at work — are brought about by such violent processes that they are undoubtedly of no significance for this process. The —S—S-bonds, which may be formed owing to oxidation of the terminal SH-groups of the cysteine residue, are of some interest. The energy content of these bonds is insignificant and the equilibrium *bond* $\rightleftharpoons$ *no bond*, the state of which is dependent on the reduction—oxidation potential, may be displaced within the cell (cf. FREY-WYSSLING, l. c., p. 128). The sulphur-containing amino-acids, however, are so scantily present (see the Tables in HOLLEMANN and RICHTER, 1944, p. 262) — it may even happen that they are completely absent in contractible molecules (silk-fibroin) — that it may be taken for granted that the sulphur bonds do not play a very important part in conditioning the variation of the shape of the spindle.

(5) Hydrogen bonds. One hydrogen atom can with fairly great power attract two atoms instead of one and may thus form a link between these two. Bonds of this kind are of very common occurrence in chemistry (cf. PAULING, 1939, p. 264, *et seq.*). In the formation of hydrogen bonds between and within the molecules the groups $—NH_2$, —OH, —COOH, =NOH, and =NH may be assumed to play a major part. Associations containing these radicals show an abnormally high

molecular weight in non-polar solvents. It would be a matter of great difficulty to establish on the basis of a roentgenographic analysis definite evidence of the existence of hydrogen bonds within protein molecules, but nevertheless it is highly probable that they exist (BRILL, 1938). In absorption spectra of light of long wave-lengths, however, the infra-red absorption band which otherwise characterizes the OH- and =NH-groups is absent, a phenomenon which, according to RODEBUSH (1936), is observed in the case of hydrated gelatin. Hydrogen bonds are assumed to play a part in the hydratation of proteins. SERRA (1942, p. 124), finally, is of the opinion that the stretching-out of polypeptide chains in the formation of the spindle is induced by the breaking-up of hydrogen and other bonds.

What are the pre-requisites encouraging the formation of hydrogen bonds within the protein molecules? As a rule, amino-acid residues include the majority of the reported groups of molecules, and the =NH-groups of the main chain have the same properties. In a sol as well as a gel the average concentration of such groups may be rather inconsiderable, but a protein molecule induces the groups to concentrate in large numbers to the area occupied by the molecule. In this area association takes place under the same conditions as in a very strong aqueous solution, as, for instance, in formic acid. Besides, an association with the help of water molecules may occur (cf. LASSETTRE, 1937).

The hypothesis that the dissociation and formation of hydrogen bonds are the forces at work in the processes of stretching and contracting the polypeptide chains has not been contradicted by any of the results of the present investigation. The hydrogen bond is no strong bond. Not more than about 5 kcal per mol are required to dissociate it, and it is of major importance (cf. PAULING, l. c., p. 264) that the activation energy of the reactions occurring in these processes is but inconsiderable. This makes it possible for dissociation and re-formation of hydrogen bonds to occur at normal temperatures. It seems as if there exists an equilibrium *hydrogen bond* $\rightleftharpoons$ *no hydrogen bond* within the entire polypeptide chain and that the state of this equilibrium is strongly dependent on temperature in a direction which is in agreement with the results of the present experiments. A rise of temperature induces dissociation of a number of hydrogen bonds, a fact resulting in a straightening of the curved outline of the spindle.

As regards the correlation between the pH and the curvature which is observed when comparing the plerome with the periblem, the conditions are more difficult to define. The equilibrium *hydrogen bond* $\rightleftharpoons$

no hydrogen bond is certainly dependent on the pH, but the relation is rather complex. In a diluted aqueous solution of, for instance, formic acid, there is certainly some association, though of an order of magnitude that is not measurable. The molecules have a bearing not only on the

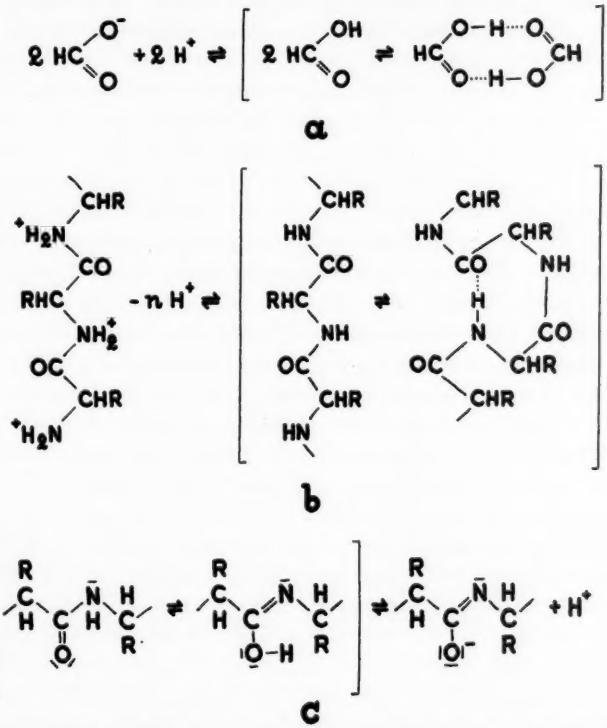

Fig. 2. *a*: pH-conditioning effect on the association of formic acid. The equilibrium within brackets is not influenced by the pH, which only controls the total amount of not ionised acid. — *b*: pH-conditioning of the hydrogen-bond equilibrium within the polypeptide main chain, if the NH-groups ionise on decreasing of pH. — *c*: pH-conditioning of the total amount of non-ionised CO-groups within the peptide chain, free to form hydrogen bonds. See text.

association but also on the dissociation equilibrium (see Fig. 2 *a*). Owing to among other factors electrostatic repulsion, two formiate ions cannot associate with the help of hydrogen bonds. An increase in the hydrogen-ion activity inducing an increase in the number of non-dissociated formic acid molecules simultaneously stimulates the formation of hydrogen bonds. In a substance with ionisable NH_2- or NH-groups the pH de-

pendence should be inverse. There is no direct causal relation between the pH variation induced if the concentration of the dissolved acid or base is modified and the simultaneous variation of the degree of association.

That the hydrogen bond equilibrium within the protein molecules depends on the pH is beyond any doubt. Hydrogen bonds may be formed between the regularly occurring =CO- and =NH-groups of the main chain (ASTBURY assumes that a linkage is formed between these groups in the transition from β-keratin to α-keratin) or between side chains. Provided the imino-groups in the main chain ionise if a fall of the pH occurs this would lead to the breaking-up of hydrogen bonds within the chain (Fig. 2 *b*). On the other hand, if it were possible to prove that a tautomeric equilibrium really exists within the main chain in accordance with Fig. 2 *c* and in which the $\equiv$C(OH)-group at stages of low hydrogen-ion activity is ionised as $\equiv CO^-$, the dependence of the hydrogen bond equilibrium on the pH would be the reverse to that which the ionising of the =NH-groups (Fig. 2 *b*) would entail. The verifying of this tautomerism would also furnish evidence that the hydrogen bonds causing the pH-conditioned molecular contraction are not formed within the main chain but chiefly between the side chains by reason of the $-NH_2$-groups of the latter being active.

Owing to the low activation energy in hydrogen-bond reactions, the equilibrium existing at a certain moment may easily be displaced. As a rule the molecules are surrounded by water, which forms hydrogen bonds with the hydrophilic groups of the protein, thus counteracting the association. If any other less hydrophilic substance having the capacity of displacing water in its quality of solvent is added, this will encourage the formation of hydrogen bonds within the protein. If substances with a colchicine effect even in weak concentrations are added to the plasma surrounding the spindle, these substances will accumulate in the direction of the polypeptide chains. In this process the VAN DER WAAL's forces discussed under (1) are at work between the lipophil groups of the C-mitotic substances and the protein molecules.

Our knowledge of the protein molecules is in many respects still defective. Regarding their composition and the properties and conditions of the hydrogen bonds we know, however, enough to make it definitely clear that hydrogen-bond reactions may occur within and between protein molecules in such a manner as to agree, at least as regards direction, with the results of the experiments reported in this

paper. It seems to the writer that the very common hydrogen bonds and, in addition, lipophil resonance forces have the greatest influence on the shape of the protein molecules and the spindle.

It should, however, be emphasized that it is extremely difficult to deny a certain type of bond all significance. Owing to the extraordinary complexity and lability of the equilibrium characterizing the living cell it is hardly possible to investigate the effect of an isolated factor. Temperature certainly has an influence on the pH, and every modified factor has an influence in the second and third line. The clarification of this phenomenon, however, will encounter great difficulty.

The curvature of the outline of the spindle is correlated to the rate of mitosis. — If it were possible to furnish evidence that there exists a direct correlation between the shape of the spindle and the speed at which the metaphase and anaphase stages are traversed, this would be an achievement of great significance for the clarification of the function of the spindle and the mechanism of mitosis. The evidence so far available, however, is rather indefinite and indirect, viz.

(1) A fall in temperature induces a greater curvature of the outline of the spindle and simultaneously the speed of anaphase is diminished.

(2) The possibility of the existence of a correlation between the growth speed of the root and the curvature of the outline of the spindle.

(3) At full C-mitosis the speed of the anaphase movement is equal to 0; the spindle is inactive. The interpolation between this condition and the normal active spindle results in a spindle with low anaphase speed and a great curvature.

Further experiments for the purpose of shedding more light on this question are in progress. Among other things, the effect of growth-stimulating substances on the shape of the spindle will be investigated.

It seems that the experience acquired in chemistry in the last ten years, especially as regards the phenomenon of tautomerism, mesomerism, and the hydrogen bonds, will be of great significance to all spheres of biology. PAULING concludes his book (1939) with the prophetic words: ». . . substances of biological importance. I have little doubt that in this field resonance and the hydrogen bond are of great significance, and that these two structural features will be found to play an important part in such physiological phenomena as the contraction of the muscle and the transmission of impulses along nerves and in the brain. . . . The attack on these substances (the proteins) by methods

of modern structural chemistry can be begun now, and it is my belief that this attack will ultimately be successful». These phenomena will most likely also be found to play an important rôle in the intriguing process termed mitosis.

The principal part of the present investigation was made at the Forest Tree Breeding Institute at Källstorp. My thanks are due to Professor NILS SYLVÉN, Director of the Institute, and his collaborators for their generous assistance, thanks to which the present investigation could be accomplished. I also wish to express my deep gratitude to my wife, Mrs. CARIN EHRENBERG, for her valuable aid and suggestions.

SUMMARY.

(1) The variation in the shape of the spindle at metaphase was studied on the basis of statistical methods and the curvature of the outline of the spindle was chosen as measurable property. The investigations were made on root meristems of *Salix fragilis* × *alba*.

(2) Changes of temperature within the range of normal temperatures condition the curvature. A rise in temperature induces a straightening of the outline of the curvature.

(3) The curvature of the outline of the spindle is greater in the plerome than in the periblem. It was also observed that the pH of the plerome cells was about 1 unit higher than that of the periblem cells.

(4) The variation in the curvature of the outline of the spindle which different roots show at one and the same temperature depends partly on the fact that the majority of the mitoses of a root occur alternately in the periblem and the plerome (pulsating periodicity), and partly most likely also on a possible correlation between the curvature and the growth speed of the root.

(5) Weak concentrations of lipophil substances in the plasma, which in strong concentrations cause dissolution of the spindle (C-mitosis), induce a curving of the outline of the spindle. So far this fact has been proved as regards ethyl alcohol and colchicine. Hydrocarbons seem to have the same tendency.

(6) It was demonstrated that the increase in the curvature of the outline of the spindle probably depended on contraction of the protein molecules, which are the main constituents of the spindle.

(7) The discussion deals with the intra- and inter-molecular forces

which may cause contraction of the polypeptide chains. The hypothesis is advanced that a variation in the number of hydrogen bonds within molecules, chiefly between =CO- and =NH-groups of the main chain, and possibly also between the side chains, causes the variation in the curvature of the outline of the spindle. Other linkages may be active too, for instance the attraction between lipophil groups within molecules, a phenomenon which, according to ÖSTERGREN (1944), may be the explanation of colchicine mitosis.

(8) It is assumed that there is a negative regression between the curvature of the outline of the spindle and the speed at which the chromosomes move at anaphase.

March 29, 1945.

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INHERITANCE OF WING DIMORPHISM IN PTEROSTICHUS ANTHRACINUS ILL.

BY *CARL H. LINDROTH*
DJURSHOLM, SWEDEN

THE non-sexual wing dimorphism seems to be of more common occurrence in the family Carabidae than in other coleopterous insects. Among the species hitherto found in Fennoscandia no fewer than 64 (i.e. 17,7 per cent) show dimorphism in this respect, at least in some part of their total distribution area (LINDROTH, 1945). Of the two forms, the macropterous one probably always possesses the power of flight, in many cases substantiated by observations, while the brachypterous one is flightless. They often show a striking difference in distribution (LINDROTH, 1939). From a purely zoogeographical point of view it would therefore be of the greatest importance to ascertain whether these characters are hereditary or not. The former opinion was advanced by DARLINGTON (1936) among others, but the opposite view has likewise found its advocates. Thus, the Finnish zoologists KLINGSTEDT (1939) and PALMÉN (1944, p. 145) have suggested that the »phase theory» of UVAROV (1921), established on the two modificatory forms of the Migratory Locust (*Locusta migratoria* L.), could be applied to cases of the type here dealt with.

The difficulties of breeding Carabids are due, among other things, to their character of being more or less pronounced predacious animals which need fresh animal food and often show cannibalism. The male, therefore, should be removed as soon as the female has been fertilized and the latter shortly after she has deposited her eggs. For one or two days, i.e. as long as she rests in the egg-cave, she will, contrary to her usual timid customs, boldly defend her offspring. But later on she devours them with a good appetite. The larvae are, to some extent, cannibal, especially about the second moulting, which within the same brood is reached at rather different ages, and hence it is better to isolate the larvae from each other in small glass tubes. Unfortunately I discovered this fact too late, which to some extent explains the small number of progeny obtained. The feeding of imagines as well as of the larvae in the case of *Pterostichus anthracinus* could be done entirely

on cut pieces of living *Lumbricus*. The substrate in the glass pots consisted of soil rich in humus (pH 4,8) from the finding place. The moisture was to some extent regulated by placing the pot at a considerable inclination. In spite of the clear hygrophilous character of the species it proved better, however, to have the moisture too low than too high. The pots were covered with loosely lying glass lids.

A special difficulty is the procuring of virginal females. Most Carabids escape as imagines in the autumn, but do not mate and develop until the next spring. The hibernation in culture represents a difficult

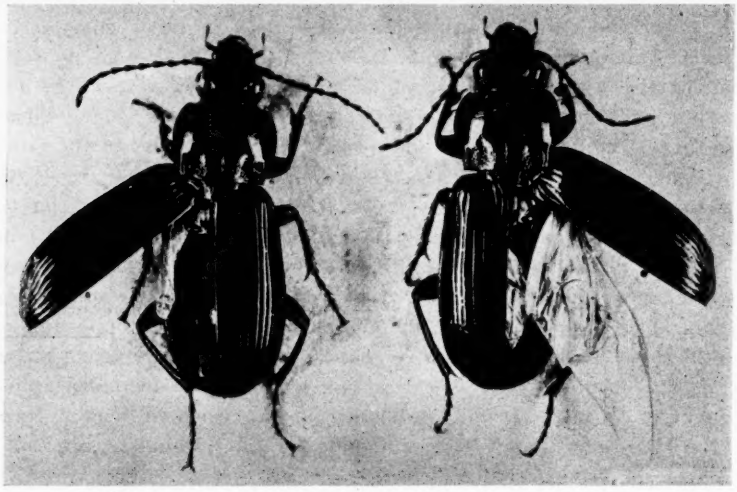

Fig. 1. Short- and long-winged female of *Pterostichus anthracinus* ILL.

task, and it was chiefly on this account that, for two years, I was without success in my attempts to make crossings with the likewise dimorphic *Bradycellus collaris* PAYK. Virginal females, however, can be obtained in the field also in spring. For the sake of control they should then be kept isolated »in quarantine» at least for so long a time as passes between copulation and oviposition, if this time is known, otherwise it must be — liberally — estimated. In the case here treated I think I have got some guarantee for the virginity of the females owing to the fact that I found them (Uppland, Djursholm, Lake Ekebysjön, April 8th and 9th, 1945) at the hibernation place under moss on rather dry ground at a distance of several metres from the place of development, the swampy lake shore. Some of the females were then kept

»in quarantine». The remaining ones deposited their eggs twenty days or more after observed copulation, a satisfactory period.

All cultures were kept indoors, in all respects under equal conditions.

Owing to the rareness of *Pterostichus anthracinus* I was not able to make more than 12 crossings (in some cases the same male was used successively with two or three different females). For different reasons merely seven cultures produced F_1 imagines, 52 specimens in all. The material obtained is thus rather small. It seems, however, as if it could be interpreted in one manner only, and so I hope there will be enough reason for its publication.

TABLE 1. *Proportions of macropterous and brachypterous offspring of Pterostichus anthracinus* ILL. *obtained in* F_1.

♀ Parent	♂ Parent	Total adult progeny	Wing condition of the progeny			
			Brachypterous		Macropterous	
			♀	♂	♀	♂
Macropterous ...	Macropterous ...	4	—	—	2	2
» ...	» [1] ...	8	—	—	6	2
» ...	» [1] ...	9	—	—	3	6
Brachypterous ...	» [1] ...	1	1	—	—	—
Macropterous ...	Brachypterous ...	2	1	—	1	—
» ...	» [2] ...	3	2	—	1	—
Brachypterous ...	» [2] ...	25	9	9	3	4
		52	13	9	16	14

[1] Mutual male. [2] Mutual male.

The results thus are as follows:

(1) Brachypterous parents may produce macropterous offspring.

(2) Macropterous parents in three crossings produced macropterous offspring only (21 specimens).

The following conclusions seem justified:

(1) The wing dimorphism of *Pterostichus anthracinus* has a hereditary base.

(2) Brachypterous wing is a dominant. The macropterous individuals are homozygotes.

(3) Inheritance takes place in a simple Mendelian fashion.

The same results and conclusions were arrived at by JACKSON

(1928) in crossings with the wing dimorphic Curculionid *Sitona hispidulus* FBR. and it therefore seems probable that the non-sexual wing dimorphism in the order of Coleoptera as a whole will prove to be of the same type.

Its greatest interest — also from a theoretical genetic point of view — possessed by this case seems to lie in the fact that it represents the instance of a mutation occurring in nature *with an unusually great power of selection.*

To these subjects I hope to be able to return in the coming part III of my treatise cited above (1945).

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DIE KOPPELUNGSGRUPPE Oh—Ar—S—Wb—K VON PISUM

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(With a Summary in English)

EINLEITUNG.

DIE fünf hier in Rede stehenden Gene haben folgende Wirkung. Das Gen *Ar* bedingt zusammen mit *A*, *B*, *Cr* und *Am* purpurfarbige Blüten. Rezessivität in *ar* verursacht blauviolette Blütenfarbe. *K* ist Bedingung für die normale Ausbildung der Blumenblätter, *k* verursacht schiffchenähnliche Umbildung der Flügel (killed wings). Das Gen *Oh* beeinflusst die Samenfarbe. *A Oh*-Samen haben gelblichbraune bis braune, *A oh*-Samen dagegen stark bräunlichrote Farbe. Das Gen *S* verursacht in rezessiver Form die Ausscheidung eines tragantähnlichen Stoffes auf der Samenschale und damit Verklebung der reifen Samen (sog. *chenille*-Merkmal). Hierfür ist jedoch Voraussetzung, dass die Samen in der Hülse so dicht sitzen, dass sie während des Reifeprozesses miteinander in Berührung stehen. Dominanz in *Wb* schliesslich ist Bedingung für die normale Ausbildung des Wachsüberzuges auf den Pflanzenteilen. Rezessivität in *wb* verursacht indessen nicht vollkommene Wachslosigkeit; nur die Hülsen sind ganz ohne Wachsüberzug, während die übrigen Teile in verschiedenem Grad schwach wachsig sind (vgl. LAMPRECHT, 1944). Eine dem Gen *wb* ganz ähnliche Wirkung hat *wa*, das jedoch im hier behandelten Material nicht spaltet. Vollkommene Wachslosigkeit wird bedingt teils durch Rezessivität im Grundgen für die Ausbildung von Wachs, *bl*, teils durch die genotypische Konstitution *wlo wsp*, d. h. zweier Gene für lokale Ausbildung von Wachs (s. l. c.).

ÜBER ZWISCHEN UND MIT DEN GENEN Oh, Ar, S, Wb UND K BISHER FESTGESTELLTE KOPPELUNGEN.

Eine Koppelung zwischen den beiden Genen *S* und *Wb* wurde schon von VILMORIN (1910, 1913) festgestellt. Seither ist dieselbe Koppelung von verschiedenen Forschern in zahlreichen Kreuzungen bestätigt worden. Unten folgt eine Übersicht der bisher mit diesen

Genen erhaltenen Spaltungen mit den dazu gehörigen Crossingoverwerten und mittleren Fehlern, berechnet nach der Methode des Produkteverhältnisses (s. IMMER, 1930; STERN, 1933).

Verfasser	Zweigenenspaltung nach Kreuzung *S Wb* × *s wb*									Cr.O.-% ± m
VILMORIN (1913)										
($F_2 + F_3$)	265	*S Wb* :		6	*S wb* :		12	*s Wb* :	57	*s wb*	5,7 ± 1,30
MEUNISSIER										
(1922)	42	»	:	2	»	:	0	» :	5	»	7,9 ± 4,07
WELLENSIEK										
(1925)	100	»	:	1	»	:	2	» :	32	»	2,2 ± 1,27
WELLENSIEK										
(1929) (3 Kr.)	2439	»	:	48	»	:	18	» :	786	»	1,8 ± 0,23
WELLENSIEK										
(1930)	782	»	:	5	»	:	4	» :	247	»	0,9 ± 0,31
LAMPRECHT										
(1939) (6 Kr.)	2012	»	:	114	»	:	119	» :	532	»	9,0 ± 0,57
Zusammen:	5640	*S Wb* :		176	*S wb* :		155	*s Wb* :	1659	*s wb*	4,5 ± 0,24

In bezug auf die Spaltungszahlen im Genpaar *S*—*s* ist folgendes zu bemerken. Wie schon einleitend erwähnt, verursacht Rezessivität im Gen *s* die Ausscheidung eines tragantähnlichen Stoffes auf der Samenschale, Verklebung der Samen aber nur dann, wenn die Samen während des Reifeprozesses miteinander in Berührung stehen. Dies hat zur Folge, dass mit dem monohybriden Verhältnis 3 *S* : 1 *s* statistisch gut übereinstimmende Spaltungszahlen nur dann erhalten werden, wenn beide Eltern in ihren Erbanlagen für dicht in den Hülsen sitzende Samen übereinstimmen. Sobald es zu Spaltung im Abstand zwischen den Samen in den Hülsen, in der Samengrösse oder in beiden diesen Merkmalen kommt, ergibt sich stets ein mehr oder weniger grosses Defizit an *s*-Individuen. Als extremes Beispiel hierfür sei meine früher veröffentlichte Kreuzung Nr. 3 angeführt (LAMPRECHT, 1936, 1939). Diese gab eine Spaltung nach 400 *S* : 56 *s*. D/m für 3 : 1 beträgt 6,3 und für 15 : 1 5,3. Diese monohybride Spaltung stimmt also infolge Nichtmanifestation eines grösseren Teils der *s*-Pflanzen sogar besser mit dem bifaktoriellen Verhältnis 15 : 1 überein. Ausgedehnte Überprüfungen der *S*-Nachkommen in F_3—F_5 haben indessen gezeigt, dass von den 87 *S wb*-Pflanzen der F_2-Generation nicht weniger als 60 die genotypische Konstitution *s* hatten. Die Nachkommen solcher Pflanzen waren allerdings zum grössten Teil wieder *S*, bildeten aber ab und zu ein oder zwei Paare miteinander verklebte Samen aus; die

Nachkommen dieser verhielten sich dann wieder ähnlich. Andere Nachkommen nach *S*-Pflanzen spalteten natürlich auch rein nach 3 *S* : 1 *s* oder verblieben konstant *S*.

Diese durch Nichtmanifestation des *s*-Merkmals bedingte fehlerhafte Klassifikation beeinflusst natürlich auch in hohem Grade die Crossingoverwerte von Zweigenenspaltungen mit *S*. So wurde in der oben als Beispiel angeführten Kreuzung Nr. 3 folgendes Spaltungsverhältnis gefunden:

313 *S Wb* : 87 *S wb* : 28 *s Wb* : 28 *s wb*

Hierfür berechnet sich ein Crossingoverwert von $31{,}7 \pm 2{,}75$ %. Aber bei Berücksichtigung, dass von den 87 *S wb*-Individuen 60 in Wirklichkeit *s* sind, ergibt sich folgendes berichtigtes bifaktorielles Verhältnis:

313 *S Wb* : 27 *S wb* : 28 *s Wb* : 88 *s wb*

Und für dieses berechnet sich ein Crossingover von $12{,}8 \pm 1{,}65$ %. Die in obiger Zusammenstellung aufgenommenen Spaltungszahlen von sechs meiner Kreuzungen sind in dieser Hinsicht berichtigt und geben dann einen mittleren Crossingoverwert von $9{,}0 \pm 0{,}57$. Die von anderen Forschern erhaltenen Zahlen sind selbstverständlich unverändert wiedergegeben. Eine der Kreuzungen von WELLENSIEK (1929) zeigt jedoch mit grösster Wahrscheinlichkeit dieselbe Erscheinung, da folgende Zahlen erhalten worden sind:

822 *S Wb* : 43 *S wb* : 9 *s Wb* : 198 *s wb*

Von den 43 *S wb*-Individuen dürfte wahrscheinlich der grössere Teil aus nur scheinbaren *S*-Individuen bestanden haben. Der oben als Mittelwert für alle bisher veröffentlichten Kreuzungen mitgeteilte Crossingoverwert von $4{,}5 \pm 0{,}24$ % wird hierdurch aber nur wenig beeinflusst.

Ausser mit *Wb* ist für das Gen *S* seit längerer Zeit auch Koppelung mit *K* festgestellt. Bisher sind folgende Spaltungen veröffentlicht.

Verfasser	Zweigenenspaltung nach Kreuzung $S\,K \times s\,k$	Cr.O.-% ± m
LAMPRECHT (1939)		
Kr. Nr. 246	301 *S K* : 56 *S k* : 38 *s K* : 70 *s k*	$22{,}4 \pm 2{,}25$
» » 261	327 » : 48 » : 34 » : 54 »	$21{,}6 \pm 2{,}21$
» » 262	324 » : 46 » : 42 » : 66 »	$22{,}3 \pm 2{,}23$
Zusammen:	952 *S K* : 150 *S k* : 114 *s K* : 190 *s k*	$21{,}9 \pm 1{,}28$

Verfasser	Zweigenenspaltung nach Kreuzung $S\,k \times s\,K$	Cr.O.-% ± m
WELLENSIEK (1930)	556 $S\,K$: 231 $S\,k$: 247 $s\,K$: 4 $s\,k$	13,7 ± 3,03

In der Koppelungsphase wurde also für *S*—*K* ein mittlerer Crossingoverwert von 21,9 ± 1,28 %, in der Repulsionsphase ein solcher von 13,7 ± 3,03 % gefunden.

Für die beiden Gene *Wb* und *K*, die auf Grund oben angeführter Kreuzungsergebnisse gleichfalls ziemlich stark gekoppelt sein müssen, sind bisher folgende Spaltungsverhältnisse veröffentlicht.

Verfasser	Zweigenenspaltung nach Kreuzung $Wb\,K \times wb\,k$	Cr.O.-% ± m
SVERDRUP (1927)	219 $Wb\,K$: 19 $Wb\,k$: 17 $wb\,K$: 61 $wb\,k$	12,2 ± 1,98
LAMPRECHT (1939)		
Kr. Nr. 246	306 » : 33 » : 60 » : 66 »	22,1 ± 2,33
» » 261	321 » : 40 » : 44 » : 58 »	21,8 ± 2,22
» » 262	331 » : 35 » : 35 » : 71 »	17,0 ± 1,93
Zusammen:	1177 $Wb\,K$: 127 $Wb\,k$: 156 $wb\,K$: 256 $wb\,k$	18,7 ± 1,06

Verfasser	Zweigenenspaltung nach Kreuzung $Wb\,k \times wb\,K$	Cr.O.-% ± m
PELLEW and SVERDRUP (1923)	374 $Wb\,K$: 174 $Wb\,k$: 164 $wb\,K$: 5 $wb\,k$	17,4 ± 3,60
WELLENSIEK (1930)	555 » : 231 » : 248 » : 4 »	13,6 ± 3,03
Zusammen:	929 $Wb\,K$: 405 $Wb\,k$: 412 $wb\,K$: 9 $wb\,k$	15,4 ± 2,32

Verfasser	Zweigenenspaltung nach Rückkreuzung $F_1 \times wb\,k$	Cr.O.-% ± m
SVERDRUP (1927)	963 $Wb\,K$: 104 $Wb\,k$: 126 $wb\,K$: 962 $wb\,k$	10,7 ± 0,67

Für die Koppelung zwischen *Wb* und *K* liegen also drei, auf verschiedene Weise erhaltene Werte vor. 1716 Individuen haben in Koppelungsphase einen Crossingoverwert von 18,7 ± 1,06 % gegeben. 1755 Individuen in Repulsionsphase 15,4 ± 2,32 % und schliesslich haben 2155 Individuen einer Rückkreuzung einen Wert von 10,7 ± 0,67 % gegeben.

Fassen wir nun die vorstehenden, bisher veröffentlichten Koppelungswerte für die drei Gene *S*, *Wb* und *K* zusammen, so ergibt sich folgendes Bild. Das Genpaar *S*—*Wb* wurde nur in der Koppelungsphase mit insgesamt 7630 Individuen untersucht und gab einen mittleren Crossingoverwert von etwa 4,5 ± 0,2 %. Erwähnt sei, dass für dieses Genpaar statistisch sicher verschiedene Koppelungswerte vorliegen. So gab z. B. WELLENSIEKs Kreuzung von 1930 mit 1038 Individuen einen Crossingoverwert von nur 0,9 ± 0,31 %, meine Kreuzung

Nr. 261 mit 463 Individuen dagegen $11{,}3 \pm 1{,}48$ % und meine bisher veröffentlichten sechs Kreuzungen (s. oben) mit zusammen 2777 Individuen einen mittleren Crossingoverwert von $9{,}0 \pm 0{,}57$ %.

Das Genpaar *S—K* gab in der Koppelungsphase mit zusammen 1406 Individuen einen Crossingoverwert von $21{,}9 \pm 1{,}28$ % und in der Repulsionsphase mit 1038 Individuen $13{,}7 \pm 3{,}03$ %. Da die mittleren Fehler dieser beiden Werte einen mathematischen Ausdruck darstellen, der sowohl die Individuenanzahl wie die methodische Sicherheit (bedingt durch Koppelungs- bzw. Repulsionsphase) berücksichtigt, kann durch Interpolation mit Hilfe dieser leicht der richtige Mittelwert ermittelt werden. Hierbei ergibt sich ein Crossingoverwert von 19,5 % für *S—K*.

Für das Genpaar *Wb—K* schliesslich liegen drei auf verschiedenen Wegen erhaltene Crossingoverwerte vor. 1716 Individuen haben in Koppelungsphase einen Crossingoverwert von $18{,}7 \pm 1{,}06$ % gegeben und 1755 Individuen in Repulsionsphase $15{,}4 \pm 2{,}32$ %. Durch Interpolation mit Hilfe der mittleren Fehler wird ein mittlerer Crossingoverwert von $17{,}7 \pm 1{,}20$ % erhalten. Nun haben überdies 2155 Individuen einer Rückkreuzung einen Crossingoverwert von $10{,}7 \pm 0{,}67$ % gegeben. Bei gleicher Berechnung mit Hilfe der mittleren Fehler ergibt sich aus diesen beiden Werten ein mittlerer Crossingoverwert für die Gene *Wb—K* von 13,2 %.

Auf Grund aller bisher vorliegenden Ergebnisse können somit die drei Gene *S*, *Wb* und *K* mit voller Sicherheit in eine Koppelungsgruppe nach folgendem Schema vereinigt werden:

$$S \leftarrow\!\!-\!\!-\!\!- 4{,}5 \!\!-\!\!-\!\!\rightarrow Wb \leftarrow\!\!-\!\!-\!\!- 13{,}2 \!\!-\!\!-\!\!\rightarrow K$$
$$S \leftarrow\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!- 19{,}5 \!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!\rightarrow K$$

Diese Koppelungsgruppe ist bisher allein dagestanden und hat mit keiner anderen mit Sicherheit vereinigt werden können. G. v. ROSEN (1944) hat allerdings einen Versuch unternommen sie an WINGES (1936) grosse Koppelungsgruppe *D—P*$_2$*—O—I—Re—Fa—Z* — — — *Uni—M—Mp—F—B—St—P*$_1$ anzuschliessen, aber seine experimentellen Unterlagen berechtigen, wie ich schon vor kurzem gezeigt habe (LAMPRECHT, 1945), keineswegs hierzu. Hierauf soll auch noch unten zurückgekommen werden.

Eine weitere, auch lange allein gestandene Koppelungsgruppe ist die zwischen den Genen *Oh* und *Ar*, die von H. und O. TEDIN (1928) gefunden worden ist. Sie erhielten folgende Spaltung:

99 *Oh Ar* : 46 *Oh ar* : 71 *oh Ar* : 0 *oh ar*

Auf Grund dieser sowie der F_3-Spaltungen ergab sich ein Crossingoverwert von etwa 12 %.

Ausser den oben besprochenen Koppelungen zwischen *S—Wb—K* und zwischen *Oh—Ar*, sind in der Literatur noch zwei weitere mitgeteilt, die sich teils auf *Ar*, teils auf *Wb* beziehen. Für das Gen *Ar* hat WINGE (1936) einwandfreie Koppelung mit dem Grundgen für Wachslosigkeit *Bl* festgestellt. In bezug auf dieses Gen hat WELLENSIEK (1928) zeigen können, dass es bei Rezessivität stets vollkommene Wachslosigkeit bedingt, welche genotypische Konstitution die Pflanzen in den übrigen Genen für die Ausbildung von Wachs auch haben mögen. Für die Spaltung in den beiden Genen *Ar* und *Bl* hat WINGE (l. c. S. 310) in drei Kreuzungen zusammen folgende Spaltungsverhältnisse erhalten:

1356 *Ar Bl* : 522 *Ar bl* : 542 *ar Bl* : 1 *ar bl*

Aus diesen Zahlen berechnet WINGE mittels zwei verschiedenen Verfahren die Crossingoverwerte 19,7 bzw. 5,1 % und als mittleren Wert 12,4 %. Bei einer Berechnung nach der Produktmethode ergibt sich $4{,}8 \pm 2{,}02$ %.

Nun ist es mir bisher leider nicht gelungen Samen von Pflanzen mit dem rezessiven Gen *bl* zu erhalten. Professor WINGE hat mir wohl in entgegenkommender Weise sein Linienmaterial, darunter auch die von ihm verwendete *bl*-Linie, seine Nr. 16, zur Verfügung gestellt. Aber diese Linie erwies sich als *wb*. Da WINGE nun zwischen *Ar* und *Bl* (d. h. *Wb*) sichere Koppelung festgestellt hat, war damit zu rechnen, dass die beiden Koppelungsgruppen *Oh—Ar* und *S—Wb—K* miteinander zu vereinigen sind, was durch die unten mitzuteilenden Kreuzungsergebnisse tatsächlich hat bestätigt werden können.

Die Koppelungsgruppe *S—Wb—K* hat v. ROSEN (1944), wie schon oben kurz erwähnt, mit WINGEs grosser Koppelungsgruppe *D—*P_2*—O—I—Re—Fa—Z — — — Uni—M—Mp—F—B—St—*P_1 zu vereinigen versucht. v. ROSEN schreibt *K—S—Wb*, obgleich auf Grund meiner Arbeit von 1939 kein Zweifel darüber bestehen konnte, dass die richtige Reihenfolge der Gene *S—Wb—K* ist. In diesem Zusammenhang verdient hervorgehoben zu werden, dass wenn man auf Grund mehrerer Kreuzungsergebnisse über die Reihenfolge von drei Genen einer Koppelungsgruppe im Zweifel sein kann, so sind stets jene Crossingoverwerte diesbezüglich entscheidend, die in Kreuzungen erhalten worden sind, in denen alle drei in Rede stehenden Gene gleichzeitig gespalten

haben. Und gerade dies war mit meinen drei l. c. besprochenen Kreuzungen Nr. 246, 261 und 262 der Fall.

In bezug auf die Gründe für die Vereinigung von *S—Wb—K* mit WINGES grosser Koppelungsgruppe sagt v. ROSEN (l. c. S. 312) folgendes: »Die Einführung der Gruppe *k—s—wb* in diesen Block ist eine Eigentümlichkeit, die nur auf Grund der neuen Daten der Artkreuzung vorgenommen worden ist. Die Vereinigung wird nur durch die 4 lokkeren Koppelungen zwischen *st—wb*, *b—k*, *b—wb* und *f—wb* (sämtliche ca. 40 %) gestützt, während ihr durch die freien Kombinationen *st—k* und *f—k* widersprochen wird.» v. ROSEN scheint also selbst etwas im Zweifel darüber zu sein, ob die in Frage stehende Vereinigung berechtigt ist, nimmt sie aber trotzdem vor. Nun sind seine Kreuzungen zwischen Kulturvarietäten von *Pisum sativum* und der geographischen Rasse *P. abyssinicum* ausgeführt, die in F_2 im Zusammenhang mit verschiedener Chromosomenstruktur nur eine durchschnittliche Fertilität von etwa 35 % gezeigt haben. Wenn in Kreuzungen etwa zwei Drittel aller Samenanlagen nicht zur Entwicklung gelangen, so erscheint wohl selbstverständlich, dass man im übrigen Drittel etwa gefundenen schwachen Koppelungen keine Beweiskraft zuerkennen kann, namentlich wenn — wie in v. ROSENs Fall — gewisse Genkombinationen direkt dagegen sprechen. Man hat ja keinerlei Anhaltspunkte dafür, welche Genotypen nicht zur Entwicklung gelangt sind. Selbst habe ich die von v. ROSEN erwähnten Genkombinationen mehrmals in normal fertilen Kreuzungen untersucht und keine Andeutung für Koppelung gefunden. Man vergleiche diesbezüglich, sowie in bezug auf WINGES oben angeführte Koppelungsgruppe Nr. 1, die mit Sicherheit in drei Koppelungsgruppen zerfällt, LAMPRECHT (1945).

DIE FESTSTELLUNG DER KOPPELUNGSGRUPPE Oh—Ar—S—Wb—K.

Ausser in meinen oben erwähnten Kreuzungen wurden die Koppelungen zwischen den verschiedenen Paaren der genannten fünf Gene in weiteren 24 Kreuzungen studiert. Eine dieser Kreuzungen, Nr. 494, spaltete in allen fünf Genen. Unten werden zuerst die Daten für diese 24 Kreuzungen sowie für drei schon früher teilweise veröffentlichte (Nr. 3, 68 und 261) kurz angeführt. Bei Angabe der genotypischen Konstitution der Elternlinien wurden nur die Gene *A*, *Ar* und *B* für Blütenfarbe sowie die übrigen vier hier in Rede stehenden Gene *Oh*, *S*, *Wb* und *K* berücksichtigt. Wo nicht besonders angegeben, haben die

Kreuzungen normale Fertilität gezeigt. Solchenfalls gehörten beide Elternlinien hinsichtlich Chromosomenstruktur dem Normaltypus an und es gab auch i. ü. keine direkt genbedingte Ausspaltung von mehr oder weniger sterilen Typen. Die nicht immer hundertprozentige Entwicklung der Samenanlagen, namentlich in höher sitzenden Hülsen, ist rein modifikativ bedingt. Gleiches gilt nicht selten auch für den Pollen in höher sitzenden Blüten. Diese letztgenannten Erscheinungen sind durch physiologische Schwäche verursacht und haben nichts mit Chromosomenstruktur oder direkt mit genotypischer Konstitution zu tun. Indirekt können natürlich gewisse Genotypen im Zusammenhang mit schwacher Vitalität auch schlechtere Ausbildung des Pollens und geringeren Prozent entwickelte Samenanlagen zeigen.

Kreuzung Nr. 3. — Linie 7 (aus Chenille), *A Ar B oh s K wb* × Linie 96 (aus Pois sabre), *a Ar B Oh S K Wb.* 500 gesäte Samen haben 456 reife, samentragende (= 91,2 %) Pflanzen gegeben.

Kreuzung Nr. 68. — Linie 7 (aus Chenille), *A Ar B oh s K wb* × Linie 110 (aus Königserbse; Roi des Gourmands), *a Ar B Oh S K Wb.* Gesät 500 Samen; erhalten 464 reife, samentragende Pflanzen = = 92,8 %.

Kreuzung Nr. 235. — Linie 11 (von Dr. H. TEDIN), *a ar b Oh S K Wb* × Linie 16 (KAJANUS' Mutante; s. H. u. O. TEDIN, 1928), *A Ar B oh S K Wb.* Linie 11 hat ein Paar reziprok translozierte Chromosomen, von denen das eine die Gene *r* und *tl* enthält. Diese Kreuzung war im Zusammenhang hiermit semisteril. Die F_1-Generation zeigte eine mittlere Fertilität von 48,4 %. Zwischen Semisterilität und der Spaltung in *ar* und *oh* bestand kein Zusammenhang. Gesät 500 Samen; erhalten 429 reife, samentragende Pflanzen = 85,8 %.

Kreuzung Nr. 237. — Linie 151 (aus Haage & Schmidts Purpurviolettschotige), *A Ar B Oh S K Wb* × Linie 232 (de Winton), *A Ar b Oh s k wb.* 500 gesäte Samen entwickelten 461 reife, samentragende Pflanzen = 92,2 %.

Kreuzung Nr. 238. — Linie 187 (*uni*-heterozygote Linie; s. LAMPRECHT, 1933), *a Ar B Oh S K Wb* × Linie 232 (de Winton), *A Ar b Oh s k wb.* 1000 gesäte Samen gaben 845 reife, samentragende Pflanzen = 84,5 %.

Kreuzung Nr. 247. — Linie 23 (aus Thüringer Erbse, *gl*), *A Ar b Oh S K Wb* × Linie 232 (de Winton), *A Ar b Oh s k wb.* 500 Samen gaben 423 reife, samentragende Pflanzen = 84,6 %.

Kreuzung Nr. 261. — Linie 15 (H. u. O. TEDINS Roterbse),

A Ar B oh S K Wb × Linie 232 (de Winton), *A Ar b Oh s k wb*. Gesät 500 Samen; erhalten 463 reife, samentragende Pflanzen = 92,6 %.

Kreuzung Nr. 301. — Linie 232 (de Winton), *A Ar b Oh s k wb* × Linie 376 (WELLENSIEKS dreiblütige), *a Ar B Oh S K Wb*. Gesät 800 Samen; erhalten 716 reife, samentragende Pflanzen = 81,4 %.

Kreuzung Nr. 352. — Linie 232 (de Winton), *A Ar b Oh s k wb* × Linie 378 (aus purpurblütiger Ackererbse; Grauerbse), *A Ar B Oh S K Wb*. 500 gesäte Samen entwickelten 441 reife, samentragende Pflanzen = 88,2 %.

Kreuzung Nr. 353. — Linie 232 (de Winton), *A Ar b Oh s k wb* × Linie 379 (aus einer Kreuzung Ambrosia I × Extra Rapid; s. LAMPRECHT, 1944), *a Ar B Oh S K Wb*. Linie 379 ist überdies im Gen *tram* rezessiv und hat im Zusammenhang hiermit Tragantflecken zwischen Samenschale und Keimblätter. Linie 379 hat ein Paar reziprok translozierte Chromosomen, von denen das eine die Gene *uni m f mp gl st* und *b* enthält (unveröffentlichtes Ergebnis). Da Linie 232 dem Normaltypus angehört, war Kr. 353 typisch semisteril. Die F_1-Generationen gaben in einem Jahr 53,0 %, in einem anderen 51,7 % Fertilität. Zwischen Semisterilität und den hier in Rede stehenden Genen gab es keinen Zusammenhang. 1500 gesäte Samen gaben 1275 reife, samentragende Pflanzen = 85,0 %.

Kreuzung Nr. 355. — Linie 232 (de Winton), *A Ar b Oh s k wb* × Linie 451 (aus Kreuzung Nr. 193: L. 176, aus Norrlandssocker × L. 205, W:s Apollo), *a Ar B Oh S K Wb*. 500 Samen gaben 451 reife, samentragende Pflanzen = 90,2 %.

Kreuzung Nr. 361. — Linie 241 (aus Goldfähnchen, *gp*), *a Ar B Oh S K Wb* × Linie 456 (aus Kr. Nr. 25: L. 5, aus Chenille × L. 102, aus Acacia), *A Ar B Oh s K wb*. Gesät 500 Samen; erhalten 385 reife, samentragende Pflanzen = 77,0 %.

Kreuzung Nr. 384. — Linie 451 (s. Kr. Nr. 355), *a Ar B Oh S K Wb* × Linie 452 (aus Kr. Nr. 25: L. 5, aus Chenille × L. 102, aus Acacia), *a Ar B Oh s K wb*. Gesät 1000 Samen; erhalten 759 reife, samentragende Pflanzen = 75,9 %.

Kreuzung Nr. 394. — Linie 232 (de Winton), *A Ar b Oh s k wb* × Linie 470 (aus Kr. Nr. 25: L. 5, aus Chenille × L. 102, aus Acacia), *a Ar B oh s K wb*. Gesät 870 Samen; erhalten 762 reife, samentragende Pflanzen = 87,6 %.

Kreuzung Nr. 432. — Linie 232 (de Winton), *A Ar b Oh s k wb* × Linie 577 (aus Kr. Nr. 209: L. 151, Haage & Schmidts Purpurviolettschotige × L. 187, *uni*-heterozygote; s. LAMPRECHT, 1933), *A Ar B Oh*

S K Wb. Gesät 1500 Samen; erhalten 1082 reife, samentragende Pflanzen = 72,2 %.

Kreuzung Nr. 433. — Linie 232 (de Winton) *A Ar b Oh s k wb* × Linie 581 (aus Air space; sehr stark panaschiert), *a Ar B Oh S K Wb.* Linie 581 hat ein Paar reziprok translozierte Chromosomen. Um welche es sich handelt, ist noch nicht festgestellt. Da Linie 232 normale Chromosomenstruktur hat, war Kr. 433 typisch semisteril. F_1 zeigte 47,8 % Fertilität. Es wurden 500 Samen gesät. Diese gaben nur 234 reife, samentragende Pflanzen = 46,8 %.

Kreuzung Nr. 449. — Linie 455 (aus Kr. Nr. 25: L. 5, aus Chenille × L. 102, aus Acacia), *a Ar B oh s K wb* × Linie 578 (aus Kr. Nr. 246: L. 19, aus H. u. O. TEDINS 0651 *z Mp* × L. 232, de Winton), *A Ar b Oh s k wb.* 1000 gesäte Samen haben sich zu 884 reifen, samentragenden Pflanzen entwickelt = 88,4 %.

Kreuzung Nr. 450. — Linie 456 (s. Kr. Nr. 361), *A Ar B oh s K wb* × Linie 577 (s. Kr. Nr. 432), *A Ar B Oh S K Wb.* Gesät 500 Samen; erhalten 257 reife, samentragende Pflanzen = 51,4 %.

Kreuzung Nr. 457. — Linie 470 (s. Kr. Nr. 394), *a Ar B oh s K wb* × Linie 581 (Air space), *a Ar B Oh S K Wb.* Da L. 581 eine reziproke Translokation enthält war diese Kreuzung semisteril; F_1 zeigte 49,8 % Fertilität. 370 Samen haben sich zu 248 reifen, samentragenden Pflanzen entwickelt = 67,0 %.

Kreuzung Nr. 473. — Linie 2 (aus Goldkönig, *o*), *a Ar B Oh S K Wb* × Linie 25 (H. TEDINS Blaublütige aus Roterbse, Rödärt), *A ar B oh S K Wb.* Linie 25 enthält eine reziproke Translokation, die aber kein Chromosom mit den hier spaltenden Genen zu treffen scheint. F_1 zeigte in einem Jahr 50,1 % Fertilität, in einem anderen 52,2 %. Gesät 485 Samen; erhalten 436 reife, samentragende Pflanzen = 90,0 %.

Kreuzung Nr. 489. — Linie 25 (s. Kr. Nr. 473), *A ar B oh S K Wb* × Linie 453 (aus Kr. Nr. 60: L. 6 aus Mummy Pea, *b fa* × L. 110, aus Königserbse; Roi des Gourmands), *A Ar b Oh S K Wb.* Da L. 25 eine reziproke Translokation enthält war die Kreuzung semisteril; F_1 zeigte 47,3 % Fertilität. 500 gesäte Samen gaben nur 276 reife, samentragende Pflanzen = 56,2 %.

Kreuzung Nr. 491. — Linie 25 (s. Kr. Nr. 473), *A ar B oh S K Wb* × Linie 482 (aus L. 453 ausgespalten; s. Kr. Nr. 489), *a Ar b Oh S K Wb.* Im Zusammenhang mit der reziproken Translokation von L. 25 war die Kreuzung semisteril. F_1 zeigte 51,4 % Fertilität. 500 Samen gesät; erhalten 423 reife, samentragende Pflanzen = 84,6 %.

Kreuzung Nr. 494. — Linie 25 (s. Kr. Nr. 473), *A ar B oh S K Wb* × Linie 578 (s. Kr. Nr. 449), *A Ar b Oh s k wb*. Da L. 25 eine reziproke Translokation enthält, war die Kreuzung typisch semisteril. F_1 zeigte 50,4 % Fertilität. Es wurden 595 Samen gesät, die sich zu 524 reifen, samentragenden Pflanzen entwickelt hatten = 88,0 %.

Kreuzung Nr. 496. — Linie 25 (s. Kr. Nr. 473), *A ar B oh S K Wb* × Linie 597 (aus Kr. Nr. 297: L. 206, W:s Olympia, *n r* × L. 369, DE HAANS *le A Ar B am Cr*), *A Ar B Oh S K Wb*. Im Zusammenhang mit der reziproken Translokation der Elternlinie 25 war die Kreuzung semisteril. F_1 zeigte 56,0 % Fertilität. Gesät 754 Samen; erhalten 451 reife, samentragende Pflanzen = 59,8 %.

Kreuzung Nr. 504. — Linie 341 (*wlo*; E. NILSSON, 1933), *a Ar B Oh S K Wb* × Linie 471 (aus Kr. Nr. 25: L. 5 aus Chenille × L. 102 aus Acacia), *a Ar B Oh s K wb*. Gesät 500 Samen; erhalten 465 reife, samentragende Pflanzen = 93,0 %.

Kreuzung Nr. 516. — Linie 453 (s. Kr. Nr. 489), *A Ar b Oh S K Wb* × Linie 566 (aus Kr. Nr. 261: L. 15, H. u. O. TEDINS Roterbse × L. 232, de Winton), *A Ar b oh s k wb*. Gesät 735 Samen; erhalten 516 reife, samentragende Pflanzen = 70,3 %.

Kreuzung Nr. 714. — Linie 458 (W:s Olympia II), *a Ar B Oh S K Wb* × Nr. 7463 (aus F_5 Kr. Nr. 384: siehe diese), *a Ar B Oh s K wb*. Gesät 500 Samen; erhalten 402 reife, samentragende Pflanzen = = 80,4 %.

Es folgt nun eine Zusammenstellung aller für die Gene *Oh*, *Ar*, *S*, *Wb* und *K* in den vorstehend angeführten 24 Kreuzungen erhaltenen Zweigenenspaltungen, wobei stets zuerst die in der Koppelungsphase und dann die in der Repulsionsphase gefundenen Zahlen angeführt werden. Anschliessend an jede solche Gruppe folgen die bisher für die betreffende Genkombination veröffentlichten Zahlen sowie schliesslich die Summen aller Spaltungen.

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Oh Ar* × *oh ar*				Cr.O.-% ± m
473	239 *Oh Ar*	6 *Oh ar*	11 *oh Ar*	73 *oh ar*	5,0 ± 1,24
489	199 »	5 »	12 »	60 »	5,8 ± 1,45
491	183 »	10 »	5 »	58 »	5,7 ± 1,50
494	374 »	26 »	33 »	91 »	12,4 ± 1,56
496	326 »	22 »	25 »	78 »	11,5 ± 1,61
Zusammen:	1321 *Oh Ar*	69 *Oh ar*	86 *oh Ar*	360 *oh ar*	8,9 ± 0,70

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Oh ar* × *oh Ar*	Cr.O.-% ± m
235	176 *Oh Ar* : 64 *Oh ar* : 66 *oh Ar* : 9 *oh ar*	36,5 ± 4,82
Bisher veröffentl. Kr.	99 » : 46 » : 71 » : 0 »	12,1 ± 2,22
Zusammen:	275 *Oh Ar* : 110 *Oh ar* : 137 *oh Ar* : 9 *oh ar*	26,2 ± 3,99

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Oh S* × *oh s*	Cr.O.-% ± m
3	286 *Oh S* : 59 *Oh s* : 54 *oh S* : 57 *oh s*	29,2 ± 2,61
68	208 » : 55 » : 51 » : 34 »	37,5 ± 3,43
450	169 » : 24 » : 37 » : 27 »	29,0 ± 3,47
516	287 » : 86 » : 84 » : 59 »	38,4 ± 2,86
Zusammen:	950 *Oh S* : 224 *Oh s* : 226 *oh S* : 177 *oh s*	34,2 ± 1,53

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Oh s* × *oh S*	Cr.O.-% ± m
261	264 *Oh S* : 80 *Oh s* : 111 *oh S* : 8 *oh s*	30,5 ± 4,16
494	394 » : 51 » : 118 » : 6 »	35,4 ± 3,76
Zusammen:	613 *Oh S* : 131 *Oh s* : 229 *oh S* : 14 *oh s*	32,9 ± 2,79

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Oh Wb* × *oh wb*	Cr.O.-% ± m
3	267 *Oh Wb* : 78 *Oh wb* : 74 *oh Wb* : 37 *oh wb*	42,6 ± 3,22
68	201 » : 62 » : 58 » : 27 »	44,2 ± 3,76
450	146 » : 47 » : 42 » : 22 »	43,2 ± 4,31
516	290 » : 83 » : 101 » : 42 »	44,8 ± 3,11
Zusammen:	904 *Oh Wb* : 270 *Oh wb* : 275 *oh Wb* : 128 *oh wb*	43,8 ± 1,76

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Oh wb* × *oh Wb*	Cr.O.-% ± m
261	266 *Oh Wb* : 78 *Oh wb* : 99 *oh Wb* : 20 *oh wb*	44,6 ± 3,69
494	299 » : 101 » : 100 » : 24 »	45,2 ± 3,44
Zusammen:	565 *Oh Wb* : 179 *Oh wb* : 199 *oh Wb* : 44 *oh wb*	45,0 ± 2,25

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Oh K* × *oh k*	Cr.O.-% ± m
516	292 *Oh K* : 81 *Oh k* : 106 *oh K* : 37 *oh k*	46,8 ± 3,18

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Oh k* × *oh K*	Cr.-O.-% ± m
261	265 *Oh K* : 80 *Oh k* : 97 *oh K* : 22 *oh k*	45,9 ± 3,64
394	338 » : 114 » : 106 » : 35 »	49,7 ± 3,09
449	379 » : 124 » : 130 » : 31 »	45,5 ± 3,04
494	287 » : 113 » : 90 » : 34 »	49,4 ± 3,30
Zusammen:	1269 *Oh K* : 431 *Oh k* : 423 *oh K* : 122 *oh k*	47,7 ± 1,62

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Ar s* × *ar S*	Cr.O.-% ± m
494	352 *Ar S* : 55 *Ar s* : 115 *ar S* : 2 *ar s*	22,2 ± 4,10

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Ar wb* × *ar Wb*	Cr.O.-% ± m
494	300 *Ar Wb* : 107 *Ar wb* : 99 *ar Wb* : 18 *ar wb*	40,5 ± 3,60
WINGE (1936)		
»*Ar bl* × *ar Bl*»	1356 » : 522 » : 542 » : 1 »	4,8 ± 2,02
Zusammen:	1656 *Ar Wb* : 629 *Ar wb* : 641 *ar Wb* : 19 *ar wb*	18,9 ± 1,76

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Ar k* × *ar K*	Cr.O.-% ± m
494	285 *Ar K* : 122 *Ar k* : 92 *ar K* : 25 *ar k*	43,5 ± 3,50

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *S Wb* × *s wb*	Cr.O.-% ± m
237	314 *S Wb* : 28 *S wb* : 55 *s Wb* : 64 *s wb*	20,0 ± 2,13
238	589 » : 24 » : 52 » : 180 »	8,7 ± 1,03
247	267 » : 34 » : 48 » : 74 »	20,6 ± 2,26
301	535 » : 29 » : 24 » : 128 »	8,2 ± 1,08
352	319 » : 18 » : 17 » : 87 »	8,5 ± 1,40
353	937 » : 50 » : 44 » : 244 »	7,9 ± 0,79
355	336 » : 15 » : 15 » : 85 »	7,2 ± 1,27
361	289 » : 14 » : 4 » : 78 »	4,2 ± 1,10
384	580 » : 28 » : 6 » : 147 »	3,7 ± 0,70
432	789 » : 49 » : 51 » : 193 »	10,2 ± 0,98
433	193 » : 7 » : 18 » : 16 »	15,3 ± 2,59
450	184 » : 22 » : 4 » : 47 »	8,3 ± 1,82
457	202 » : 3 » : 3 » : 40 »	2,8 ± 1,06
494	394 » : 73 » : 5 » : 52 »	10,5 ± 1,43
504	355 » : 4 » : 4 » : 102 »	1,8 ± 0,63
516	352 » : 41 » : 39 » : 84 »	17,2 ± 1,86
714	310 » : 4 » : 4 » : 84 »	2,1 ± 0,72
Bisher veröffentl. Kr.	5640 » : 176 » : 155 » : 1659 »	4,5 ± 0,24
Zusammen:	12.585 *S Wb* : 619 *S wb* : 548 *s Wb* : 3364 *s wb*	7,3 ± 0,22

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *S K* × *s k*	Cr.O.-% ± m
237	302 *S K* : 40 *S k* : 47 *s K* : 72 *s k*	21,0 ± 2,18
238	537 » : 76 » : 79 » : 153 »	19,6 ± 1,56
247	271 » : 40 » : 51 » : 61 »	24,3 ± 2,47
301	313 » : 24 » : 34 » : 61 »	15,8 ± 1,91
352	305 » : 22 » : 39 » : 65 »	18,4 ± 2,08
353	897 » : 155 » : 79 » : 144 »	21,8 ± 1,34

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *S K* × *s k*	Cr.O.-% ± m
355	325 *S K* : 38 *S k* : 34 *s K* : 54 *s k*	19,7 ± 2,13
432	750 » : 88 » : 98 » : 146 »	20,2 ± 1,40
433	167 » : 33 » : 12 » : 22 »	23,0 ± 3,21
494	359 » : 108 » : 18 » : 39 »	25,4 ± 2,26
516	336 » : 57 » : 62 » : 61 »	27,7 ± 2,39
Bisher veröffentl. Kr.	952 » : 118 » : 114 » : 222 »	18,5 ± 1,17
Zusammen:	5514 *S K* : 809 *S k* : 667 *s K* : 1100 *s k*	21,3 ± 0,54

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *S k* × *s K*	Cr.O.-% ± m
Bisher veröffentl. Kr.	556 *S K* : 231 *S k* : 247 *s K* : 4 *s k*	13,7 ± 3,03

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Wb K* × *wb k*	Cr.O.-% ± m
237	319 *Wb K* : 50 *Wb k* : 30 *wb K* : 62 *wb k*	19,9 ± 2,12
238	551 » : 90 » : 65 » : 139 »	20,0 ± 1,57
247	284 » : 41 » : 38 » : 60 »	21,5 ± 2,30
301	312 » : 25 » : 35 » : 61 »	16,0 ± 1,95
352	307 » : 29 » : 37 » : 68 »	16,9 ± 1,99
353	874 » : 107 » : 102 » : 192 »	18,7 ± 1,23
355	326 » : 25 » : 33 » : 67 »	14,8 ± 1,84
432	755 » : 85 » : 93 » : 149 »	19,3 ± 1,36
433	175 » : 36 » : 4 » : 19 »	15,7 ± 2,63
494	343 » : 56 » : 34 » : 91 »	18,2 ± 1,89
516	348 » : 43 » : 50 » : 75 »	20,6 ± 2,04
Bisher veröffentl. Kr.	1177 » : 127 » : 156 » : 256 »	18,7 ± 1,06
Zusammen:	5761 *Wb K* : 714 *Wb k* : 677 *wb K* : 1239 *wb k*	19,0 ± 0,48

Kreuzung Nr.	Zweigenenspaltung nach Kreuzung *Wb k* × *wb K*	Cr.O.-% ± m
Bisher veröffentl. Kr.	929 *Wb K* : 405 *Wb k* : 412 *wb K* : 9 *wb k*	15,4 ± 2,32

Kreuzung Nr.	Zweigenenspaltung nach Rückkreuzung von F_1 *Wbwb Kk* × *wb k*	Cr.O.-% ± m
SVERDRUP (1927)	963 *Wb K* : 104 *Wb k* : 126 *wb K* : 962 *wb k*	10,7 ± 0,67

Es sollen nun die vorstehenden Kreuzungsergebnisse zur Klarlegung der Beziehungen zwischen den Genen *Oh*, *Ar*, *S*, *Wb* und *K* ausgewertet werden. Die Feststellung der Reihenfolge zwischen meh-

reren gekoppelten Genen auf Grund der Spaltungen in verschiedenen Kreuzungen, in denen nur je ein Paar Gene spaltet, stösst häufig auf Schwierigkeiten. Verursacht wird dies dadurch, dass man für den Crossingoverprozent in verschiedenen Kreuzungen mehr oder weniger abweichende Werte erhalten kann. Ein solcher variierender Grad von Koppelung wurde mehrmals festgestellt. Man vergleiche z. B. für *Pisum* RASMUSSON (1927) und LAMPRECHT (1939), für *Phaseolus* LAMPRECHT (1932). Die oben mitgeteilten Zahlen bilden diesbezüglich auch ein lehrreiches Material. Am stärksten tritt diese Erscheinung des wechselnden Koppelungsgrades zwischen stark gekoppelten Genen, wie *Oh—Ar* und besonders *S—Wb* zutage. Besonders stark scheinen in der Repulsionsphase erhaltene Zahlen schwanken zu können.

So wurden für das Genpaar *Oh—Ar* in der Koppelungsphase Werte von $5{,}0 \pm 1{,}24$ bis $12{,}4 \pm 1{,}56$ % Crossingover, in der Repulsionsphase ein solcher von $12{,}1 \pm 2{,}22$ und von $36{,}5 \pm 4{,}82$ % gefunden. In beiden Phasen erscheinen die extremen Werte statistisch sicher verschieden. Für die Koppelungsphase erhält man für $D/m_{Diff.}$ zwischen 5,0 und 12,4 einen Wert von 3,72 und für die beiden Werte der Repulsionsphase einen solchen von sogar 4,59. Noch grösser sind die Unterschiede für die beiden stark gekoppelten Gene *S* und *Wb*. Für die beiden bisher am stärksten verschiedenen Crossingoverwerte, WELLENSIEKs Kreuzung von 1930 mit $0{,}9 \pm 0{,}31$ % einerseits sowie meine Kreuzung Nr. 247 mit $20{,}6 \pm 2{,}26$ % andererseits ergibt sich ein $D/m_{Diff.}$ von nicht weniger als 8,63. Die Erscheinung des variierenden Koppelungsgrades soll jedoch hier nicht weiter erörtert sondern einer besonderen Untersuchung vorbehalten bleiben.

Die angeführten, für dasselbe Genpaar zuweilen stark wechselnden Crossingoverwerte zeigen, mit welcher Unsicherheit es verknüpft sein kann, die Reihenfolge von Genen einer Koppelungsgruppe auf Grund von Genpaarspaltungen in verschiedenen Kreuzungen festzustellen. Wie schon oben erwähnt, geht man viel sicherer, wenn Kreuzungen studiert werden, in denen drei oder noch besser mehrere Gene einer Koppelungsgruppe gleichzeitig spalten. Die oben mitgeteilten Spaltungsergebnisse werden daher unten vorerst auf vier Gruppen verteilt behandelt. Gruppe 1): 9 Kreuzungen mit gleichzeitiger Spaltung in den Genen *S*, *Wb* und *K*; Gruppe 2): Zwei Kreuzungen mit gleichzeitiger Spaltung in den Genen *Oh*, *S* und *Wb*; Gruppe 3): Zwei Kreuzungen mit gleichzeitiger Spaltung in den vier Genen *Oh*, *S*, *Wb* und *K* sowie Gruppe 4): Eine Kreuzung, in der alle fünf Gene der hier in Frage stehenden Koppelungsgruppe spalten.

Gruppe 1. — Die Crossingoverwerte von in *S*, *Wb* und *K* spaltenden Kreuzungen.

Kr. 237: $S \leftarrow 20{,}0 \rightarrow Wb \leftarrow 19{,}9 \rightarrow K$
$S \longleftarrow 21{,}0 \longrightarrow K$

Kr. 238: $S \leftarrow 8{,}7 \rightarrow Wb \leftarrow 20{,}0 \rightarrow K$
$S \longleftarrow 19{,}6 \longrightarrow K$

Kr. 247: $S \leftarrow 20{,}6 \rightarrow Wb \leftarrow 21{,}5 \rightarrow K$
$S \longleftarrow 24{,}3 \longrightarrow K$

Kr. 301: $S \leftarrow 8{,}2 \rightarrow Wb \leftarrow 16{,}0 \rightarrow K$
$S \longleftarrow 15{,}8 \longrightarrow K$

Kr. 352: $S \leftarrow 8{,}5 \rightarrow Wb \leftarrow 16{,}9 \rightarrow K$
$S \longleftarrow 18{,}4 \longleftarrow K$

Kr. 353: $S \leftarrow 7{,}9 \rightarrow Wb \leftarrow 18{,}7 \rightarrow K$
$S \longleftarrow 21{,}8 \longrightarrow K$

Kr. 355: $S \leftarrow 7{,}2 \rightarrow Wb \leftarrow 14{,}8 \rightarrow K$
$S \longleftarrow 19{,}7 \longrightarrow K$

Kr. 432: $S \leftarrow 10{,}2 \rightarrow Wb \leftarrow 19{,}3 \rightarrow K$
$S \longleftarrow 20{,}2 \longrightarrow K$

Kr. 433: $S \leftarrow 15{,}3 \leftarrow Wb \leftarrow 15{,}7 \rightarrow K$
$S \longleftarrow 23{,}0 \longrightarrow K$

Von den Crossingoverwerten der obigen neun Kreuzungen sprechen die von vier, Nr. 247, 352, 353 und 355, für die Reihenfolge *S*—*Wb*—*K*, während die der übrigen fünf Kreuzungen ungefähr gleich gut mit der Reihenfolge *Wb*—*S*—*K* übereinstimmen. Da aber keines der Ergebnisse direkt für die letztgenannte Reihenfolge spricht, ist die Reihenfolge *S*—*Wb*—*K* als die richtige zu betrachten. Die nächsten Gruppen werden dies einwandfrei bestätigen.

Gruppe 2. — Die Crossingoverwerte von zwei in *Oh*, *S* und *Wb* spaltenden Kreuzungen.

Kr. 3: $Oh \leftarrow 29{,}2 \rightarrow S \leftarrow 12{,}8 \rightarrow Wb$
$Oh \longleftarrow 42{,}6 \longrightarrow Wb$

Kr. 450: $Oh \leftarrow 29{,}0 \rightarrow S \leftarrow 8{,}3 \rightarrow Wb$
$Oh \longleftarrow 43{,}2 \longrightarrow Wb$

Die Spaltungsergebnisse dieser beiden Kreuzungen können wie ersichtlich nur in einer Weise gedeutet werden. Zusammen mit den Ergebnissen der neun Kreuzungen von Gruppe 1) beweisen sie die Koppelungsgruppe *Oh*—*S*—*Wb*—*K*. (Der Crossingoverwert für die Koppelung *S*—*Wb* von Kreuzung Nr. 3 ist, da schon früher veröffentlicht, in den Zahlen für »Bisher veröffentlichte Kreuzungen» enthalten.)

Gruppe 3. — Die Crossingoverwerte von zwei in den vier Genen *Oh*, *S*, *Wb* und *K* spaltenden Kreuzungen. (Die Zahlen für die Koppelung der Gene *S*, *Wb* und *K* von Kr. 261 sind oben in denjenigen für »Bisher veröffentl. Kr.» enthalten.)

Kr. 261: $Oh \longleftarrow 30{,}5 \longrightarrow S \longleftarrow 11{,}3 \longrightarrow Wb \longleftarrow 21{,}8 \longrightarrow K$
$Oh \longleftarrow 44{,}6 \longrightarrow Wb$
$S \longleftarrow 22{,}4 \longrightarrow K$
$Oh \longleftarrow 45{,}9 \longrightarrow K$

Kr. 516: *Oh*←38,4→*S*←17,2→*Wb*←20,6→*K*
Oh←44,8→*Wb*
S←27,7→*K*
Oh←46,8→*K*

Die Spaltungen dieser beiden Kreuzungen bestätigen des weiteren die Koppelungsgruppe *Oh—S—Wb—K*, die schon durch die Werte der »Gruppe 2» einwandfrei bewiesen worden ist. Dass zu dieser Gruppe auch das Gen *Ar* gehört, wird durch die Ergebnisse von sieben in der obigen Zusammenstellung aufgenommenen Kreuzungen dargetan, die für *Oh—Ar* in der Koppelungsphase einen Crossingoverwert von 8,9 % und in der Repulsionsphase einen solchen von 26,2 % gegeben haben. Das Gen *Ar* liegt also wahrscheinlich zwischen *Oh* und *S*.

Gruppe 4. — Die Crossingoverwerte der in allen fünf Genen der in Frage stehenden Koppelungsgruppe spaltenden Kreuzung Nr. 494.

Oh←12,4→*Ar*←22,2→*S*←10,5→*Wb*←18,2→*K*
Oh←35,4→*S*←25,4→*K*
Ar←40,5→*Wb*
Oh←45,2→*Wb*
Ar←43,5→*K*
Oh←49,4→*K*

Damit kann diese Koppelungsgruppe von fünf Genen sowohl hinsichtlich Reihenfolge der Gene wie ungefähre Abstände zwischen diesen als klargelegt betrachtet werden. Wie schon früher hervorgehoben wurde, sind die Ergebnisse einer Kreuzung, in der gleichzeitig mehrere Gene einer Koppelungsgruppe spalten nicht nur für die Feststellung der Reihenfolge, sondern vor allem in bezug auf die Abstände zwischen den Genen viel sicherer als die durch Kombination einzelner Genpaarspaltungen verschiedener Kreuzungen erhältlichen Werte. Im letzteren Fall bedarf es mit Hinblick auf den ziemlich stark variierenden Koppelungsgrad eines grossen Materials um sichere Werte zu erhalten. Zum Vergleich seien hier noch die auf Grund des gesamten Materials berechneten mittleren Werte für diese Koppelungsgruppe mitgeteilt:

Oh←11,4→*Ar*←22,2→*S*←7,3→*Wb*←14,9→*K*
Oh←33,7→*S*←20,1→*K*

Wie ersichtlich stimmen diese Werte gut mit den in Kreuzung Nr. 494 (s. oben Gruppe 4) erhaltenen überein, die in allen fünf Genen spaltete.

Ein Blick auf die in den einzelnen Kreuzungen erhaltenen Werte (s. die Zusammenstellung) zeigt dagegen, dass man durch Kombination der Koppelungswerte einzelner Kreuzungen, wenigstens in bezug auf die Genenabstände, zu stark abweichenden Ergebnissen kommen kann. Diese Erscheinung hängt mit der teils durch Umweltverhältnisse (Temperatur, Feuchtigkeit usw.), teils durch die genotypische Konstitution bedingten Variation der Koppelungswerte zusammen. Für die Klarlegung dieser Variation und ihrer Ursachen sind besondere Versuche mit Rückkreuzungen in grösserem Umfang erforderlich.

SUMMARY.

(1) The hitherto published results on linkage between the genes *S*, *Wb* and *K* as well as *Oh* and *Ar* respectively and the relations of these two linkage groups to other genes are critically studied.

(2) In 24 new crosses resulting in about 15,000 individuals it has been found that the five genes *Oh—Ar—S—Wb—K* belong to one linkage group, with the genes in the succession mentioned.

(3) The linkage between *Ar* and *Bl*, stated by WINGE (1936), is referred to *Ar* and *Wb*. G. v. ROSEN's (1944) attempt to append *S—Wb—K* to WINGE's large linkage group *D—P_2—O—I—Re—Fa—Z — — — Uni—M—Mp—F—B—St—P_1* is experimentally unfoundèd and could not be confirmed.

(4) The crossing-over values, especially between the more strongly linked genes, show a rather great variation.

(5) It is shown that reliable crossing-over values are obtainable in crosses which segregate in at least three or preferable more genes of the same linkage group.

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STRUCTURAL CHANGES OF THE Y CHROMOSOME IN THE OFFSPRING OF POLYPLOID MELANDRIUM

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(Preliminary report)

THE dioecious plant *Melandrium album* has distinct heteromorphic sex chromosomes, the *Y* chromosome is longer than the *X* chromosome and has a median centromere, whereas *X* has a submedian centromere (BLACKBURN, 1923; WARMKE and BLAKESLEE, 1939; WESTERGAARD, 1940). Recent studies on sex expression in experimentally produced polyploids have furnished important information on the mechanism of sex determination and genetical structure of the sex chromosomes (ONO, 1940; WARMKE and BLAKESLEE, 1939; WESTERGAARD, 1940), the *Y* chromosome being male-determining, whereas the female-determining element is located in the autosomes and the *X* chromosome. The associations of the *Y* chromosomes in *XXYY* males show a very low chiasma frequency in the differential arms of the *Y* chromosomes.

In the offspring of polyploid *Melandrium* the *Y* chromosome is found to undergo spontaneous structural changes, and three well differentiated types have so far been observed. Two of these have probably arisen through terminal fragmentations, one in the homologous and the other in the differential arm of the *Y* chromosome. The third type has either arisen through a terminal fragmentation of the differential arm of the *Y* chromosome or, more probably, by a reciprocal translocation between the differential arms of the *X* and *Y* chromosomes. The three new chromosomes are designated Y^1, Y^2 and Y^3 and behave as follows.

Y^1. — The Y^1 chromosome is slightly longer than the *X* chromosome, it has probably arisen through a terminal fragmentation of the differential arm of the *Y* chromosome, the length of the lost fragment measuring slightly less than $^2/_3$ of the differential arm. The type has appeared three times in the material. Plants of the constitution $XXXY^1$ are intersexual and on selfing produce ♀♀ and ♂♀ in an approximate ratio of 25: 75; the ♂♀ include $XXXY^1$ and XXY^1Y^1 plants, which cannot be distinguished morphologically. The latter type gives rise to

♀♀ and ♂♀ in a proportion of about 7: 100. Among the intersexes one plant was found with the chromosome constitution $XY^1Y^1Y^1$. The differential arm of the Y^1 chromosome never associates with X, and therefore the same types of associations are formed as previously described in the $XXXY$ males (WESTERGAARD, 1940). In the XXY^1Y^1 ♂♀ the associations of the Y^1Y^1 chromosomes show a very low chiasma frequency in the differential arms, just as was found in the $XXYY$ males.

Y^2. — The Y^2 chromosome is slightly shorter than the X chromosome, the change involves the differential arm only which is about $^1/_4$ of the normal length. This type, too, may have arisen through a simple fragmentation of the differential arm; however, the behaviour of the chromosome during meiosis makes it more probable that a reciprocal translocation has taken place between a fairly short segment of the differential arm of the X chromosome and a long section of the differential arm of the Y chromosome. The type has appeared twice in the material. Plants having the sex chromosome constitution $XXXY^2$ are ♂♀ and produce ♀♀ and ♂♀ on selfing, the proportion again being approximately 25: 75. The latter group includes $XXXY^2$ and XXY^2Y^2 plants as in the first case. On selfing the XXY^2Y^2 intersexes a great excess of ♂♂ are found in the offspring. *Cytologically, the Y^2 chromosome differs in a remarkable way from the Y^1 type in forming chiasmata with the differential arm of the X chromosome in $XXXY^2$* ♂♀. The chiasma frequency amounts to about 55 per cent. In the homozygous XXY^2Y^2 ♂♀ the associations of the Y^2 chromosomes show that the chiasma frequency is high (about 40 per cent) in the differential arm in contradistinction to the conditions found in the Y and Y^1 chromosomes.

Y^3. — This chromosome is longer than Y^1 and Y^2 and has probably arisen through a terminal fragmentation in the homologous section of Y, the fragment being slightly less than half the length of the arm. The type has appeared only once. Plants containing the Y^3 chromosome are generally males, but they are pollen-sterile, the anthers stopping development at a fairly late stage. Fortunately, the first plant was slightly intersexual, probably because of its autosome constitution, and it could be propagated by outcrossing to normal males. The same type seems to have been described recently in American strains of *Melandrium* (WARMKE and DAVIDSON, 1944), in which the homologous arm of the Y chromosome apparently undergoes different fragmentations with a high frequency. In $XXXY^3$ plants the Y^3 cannot associate

with any of the arms of the *X* chromosome; hence it is always found as a univalent during metaphase I.

The difference in behaviour of the three *Y* chromosomes during meiosis is demonstrated very distinctly when intersexual plants of the constitution $XXXY^1$, $XXXY^2$ and $XXXY^3$ are pollinated with normal diploid *XY* males. The cross $XXXY^1 \times XY$ gives rise to *XXX* ♀♀, XXY^1 ⚥⚥, *XXY* ♂♂ and XYY^1 ♂♂. From the cross $XXXY^2 \times XY$ are likewise produced *XXX* ♀♀, XXY^2 ⚥⚥, *XXY* ♂♂ and XYY^2 ♂♂. Finally, the third combination results in the production of *XXX* ♀♀, XXY^3 sterile ♂♂, *XXY* ♂♂ and XYY^3 ♂♂. A cytological comparison between the XYY^1, XYY^2 and XYY^3 males shows that in the XYY^1 plants one type only of trivalents is observed during metaphase I $\left(\begin{matrix} Y^1 \\ X \end{matrix}\!\!>\!\!-\!\!-\!\!-Y\right)$, the formation of which requires a triple chiasma between the three homologous arms. The trisome is observed in about 40 per cent of the cells. In the other cells a bivalent $X - Y$, $X - Y^1$ and $Y - Y^1$ with univalent Y^1, *Y* and *X* respectively is formed. This proves that the differential arm of the Y^1 chromosome does not associate with either the differential arm of *X* or with that of *Y*. In the XYY^2 males, trivalents are found in 90 per cent of the cells and three different types are formed, viz. $Y^2 - X - Y$, $\begin{matrix} Y^2 \\ X \end{matrix}\!\!>\!\!-\!\!-Y$ and less frequently $\begin{matrix} Y^2 \\ X \end{matrix}\!\!>\!\!-\!\!-Y$. These types are formed because Y^2 associates with the differential arm of *X*, but not with the differential arm of *Y*. Therefore the associations are of the same types as previously described in *XXY* ♂♂ (WESTERGAARD, 1940). In the XYY^3 males a bivalent $X - Y$ and a univalent Y^3 chromosome are formed almost exclusively. In a very few cases, however, a chain of the constitution $X - Y - Y^3$ may be seen. This agrees with the low chiasma frequency found in the differential arms of the *Y* chromosomes in *XXYY* plants.

The present material gives some very interesting informations about the composition of the male-determining element in the *Y* chromosome of *Melandrium*. It is now possible to distinguish between two (or three) different genes (or gene complexes) which must be present in order to produce normal males. First, a gene (or genes) is located in the distal end of the differential arm, the presence of which suppresses the development of the female organs. When the distal segment is lost (in the Y^1 and Y^2 plants) the female organs are developed. However, plants containing Y^1 and Y^2 chromosomes are still intersexual. Therefore a second gene (or gene complex) for the devel-

opment of the male organs, the anthers, must still be present. This second gene must be located in the proximal part of the differential section of the *Y* chromosome, and if the translocation hypothesis of the origin of the Y^2 chromosome is adopted it should probably be located in the region between the centromere and the homologous section (cf. WESTERGAARD, 1940, Fig. 33, p. 75). Finally, the Y^3 type indicates the presence of a gene (or genes) for pollen sterility, probably located in the homologous segment of the *Y* chromosome. However, the possibility cannot be excluded that this gene, too, is actually situated in the differential segment if the homologous segment of the sex chromosomes is shorter than the lost fragment. In fact, it has been hitherto suggested that the length of the homologous sections corresponds to the length of the longer arm of the *X* chromosome, but some new observations indicate that it may actually be shorter. Whether or not genes for pollen sterility should be called sex-determining genes (cf. WARMKE and DAVIDSON, 1944) is of course a matter of definition. In the author's opinion, however, it should be most practical to confine the term »sex-determining genes» to those genes which according to their mode of action must be located in the differential segments of the sex chromosomes where crossing over is suppressed. In *Melandrium* a stable sex-determining mechanism is only possible when the genes suppressing the development of female organs and the genes for the production of male organs are located in the differential section, whereas the genes for pollen sterility may very well be located in the homologous segment or in the autosomes because they will only be able to function when the genes in the differential arm are present.

It should be pointed out that, according to unpublished material of the author, a fairly great number of intersexual plants, containing one or two normal *Y* chromosomes and a varying number of *X* chromosomes and autosomes and exhibiting various degrees of intersexuality, are produced in the offspring of triploid plants. The formation of these intersexes must be due to an accumulation of the female-determining genes located in the *X* chromosomes and the autosomes, such an accumulation being possible because of the special mode of segregation of the autosomes and *X* chromosomes in the triploid $3A + XXY$ plants. The occurrence of these intersexes shows that the gene in the distal part of the *Y* chromosome which suppresses the formation of female organs is not absolutely epistatic to the female-determining element. However, the gene or gene complex for the formation of male organs, which is located in the proximal part of the differential segment

of Y, seems to be absolutely epistatic to the female-determining element, because plants containing Y^1 and Y^2 chromosomes are always intersexual irrespective of the number of autosomes and X chromosomes. At the same time the presence of this segment seems to be absolutely necessary for the developing of the male organs, since hitherto no intersexual plants have been detected containing X chromosomes and autosomes alone.

Finally it should be pointed out that several problems still call for further cytological and genetical investigations before the sex-determining mechanism of *Melandrium* can be regarded as thoroughly explained. For instance, the low chiasma frequency in the differential arms of the Y and Y^1 chromosomes in $XXYY$ and XXY^1Y^1 plants is still unexplained; the hypothesis previously suggested by the present author must be abandoned in the light of the present material.

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THE INFLUENCE OF LOW TEMPERATURES ON THE DEVELOPMENT OF THE EMBRYO-SAC MOTHER CELL IN LILIUM LONGIFLORUM THUNB.

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THE object of this paper is to give an account of some investigations into the development of the meiosis and related phenomena in the ovules of *Lilium longiflorum* THUNB., apparently induced by low temperatures. In explanation of the somewhat vague statements made as to the cause of the deviations from normal development in the material investigated I must premise that this material was intended for the preparation of slides for the demonstration to students of the E.M.C. development in *Lilium*. It was soon discovered that instead of illustrating the normal course of meiosis most of the slides showed quite a different picture. The material happened to be fixed during the winter, under extremely cold conditions, sometimes with only $+ 5^\circ$ C. or less in the greenhouses.

I mention this because the experimental treatment of my material cannot be considered to meet the demands which ought to be exacted of experiments intended to elucidate the control of meiosis by external conditions. In that respect, STRAUB's (1939) *Gasteria* investigations may be cited as worthy of being taken as a pattern. However, I consider that the results obtained from my material are of interest, since, in certain respects, they reveal very peculiar conditions, which, in spite of the less satisfactory experimental dispositions, may be considered to have a connexion with the temperature control of meiotic phenomena and perhaps to some extent illustrate the relation between mitosis and meiosis.

So far, the material investigated for the study of the influence of temperature on meiosis has generally consisted of P.M.C's, more rarely of E.M.C's. However, it is not *a priori* certain that the reaction is the same (cf. STEBBINS, 1941). And the embryo-sac in *Lilium* is tetrasporic, which means that besides meiosis a further three nuclear divisions take place in the E.M.C., the course of which under the given conditions may be of interest to follow. The P.M.C. is the more suitable to study because in a *Lilium* stamen there are a large number of pollen mother-

cells in the same compartment, whereas in the ovule there is only one E.M.C. The ovules, however, are numerous and close together and horizontally oriented in the ovary. Another advantageous property is that they present rather different stages of development in a rather distinctly appearing basipetal sequence of development. Hence, as a general rule the ovules in the upper part of the ovary are more developed than in the lower part, although a small number of ovules high up in the ovary show a somewhat retarded development.

In the present *Lilium* material, however, the order is disturbed by the fact that as a result of the low temperature here and there the meiosis of an E.M.C. is suppressed or retarded, so that ovules can be found with a nucleus in the pachytene phase strewn among ovules with 8-nucleated embryo-sacs.

MITOTIC DIVISIONS IN THE EMBRYO-SAC MOTHER CELL.

In *Lilium* an embryo-sac mother cell is very early constituted by the enlargement of a subepidermal cell. This turns direct into the embryo-sac, and the first division is meiotic, after which three other mitotic divisions follow. Two of the chromosomes are bigger, with submedian centromeres. In very young ovaries of my material it often appeared as if the nucellus had several archesporial cells instead of only a single one. A closer examination, however, gave the explanation, it being found that in several ovules the E.M.C. nucleus divided mitotically into two or more cells separated by transverse or longitudinal walls. Fig. 1 *e*, *f* shows different forms of this process of division, reminiscent of polymitoses in the pollen cells of *Sorghum* (DARLINGTON and THOMAS, 1941). Further details can be seen in Figs. 3 and 4. There is no doubt as to its actually being an E.M.C. that has divided, for, towards the chalazal region, this always adjoins a number of more or less elongated, palisade-shaped cells, which manifestly may facilitate the transport of nourishment to the E.M.C. (Fig. 1 *e*, *f*; Fig. 3 *d*).

Fig. 1 *a* shows an early stage with the nucleus most closely in leptotene. At this stage there always appears in the plasm a filamentous structure and, in addition, a vacuole that is mostly absent at this stage in material unaffected by cold. Fig. 1 *b* and *d* shows a prophase stage, which however deviates sharply from a meiotic prophase. The number of the chromosomes is 24, a fact that I was easily able to verify on several occasions. Here and there, though more rarely, stages are

encountered like that in Fig. 1 *c*, which shows a distinct mitotic metaphase.

A number of detailed views under strong magnification are submitted in Fig. 2. Fig. 2 *d* shows some chromosomes that are clearly in prophase. In later stages the coiling is especially distinct. In figures *f* and *h* a nucleolar chromosome is seen. The figures do not show all the chromosomes in the nucleus, but most of those illustrated are whole chromosomes, not cut ones. In a couple of cases a clear staining of the

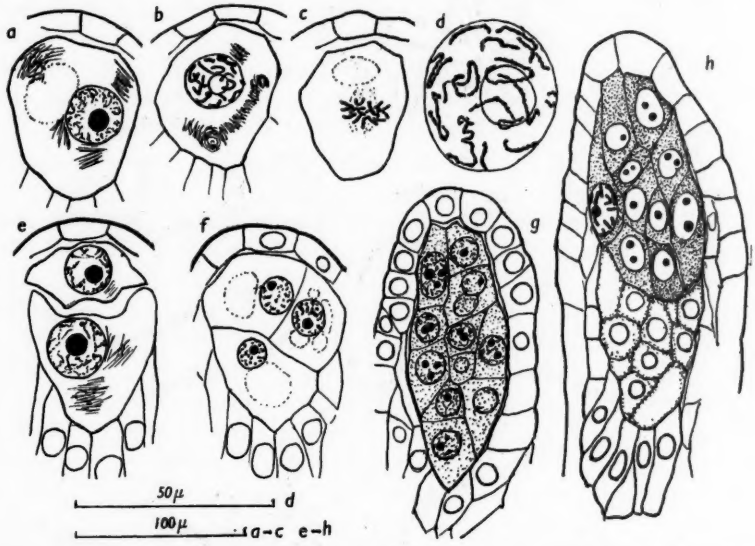

Fig. 1 *a—d*: Mitotic division in the E.M.C., *a*: resting stage, cytoplasm with vacuoles and well-developed filamentous structure, *b*: prophase, *c*: metaphase, *d*: part of prophase under higher magnification (24 chromosomes in the whole nucleus), *e—h*: cell-complexes derived from mitotic divisions of the E.M.C.; in *h* this complex has developed into an upper part with dense cytoplasm and a lower part with vacuoles.

chromosome spiral including the adjacent cortex could be fairly successfully obtained with haematoxylin and orange G in clove oil. For comparison an illustration is given in Fig. 2 *b* and *c* of a late prophase in clear mitosis in one of the cells of the cell-body that had arisen through mitotic division of the E.M.C. nucleus. Fig. 2 *i* illustrates a prophase immediately preceding metaphase. The chromosomes then finally arrange themselves in a more or less normal mitosis. To interpret the two last stages as asynapsis in the sense that this nuclear division

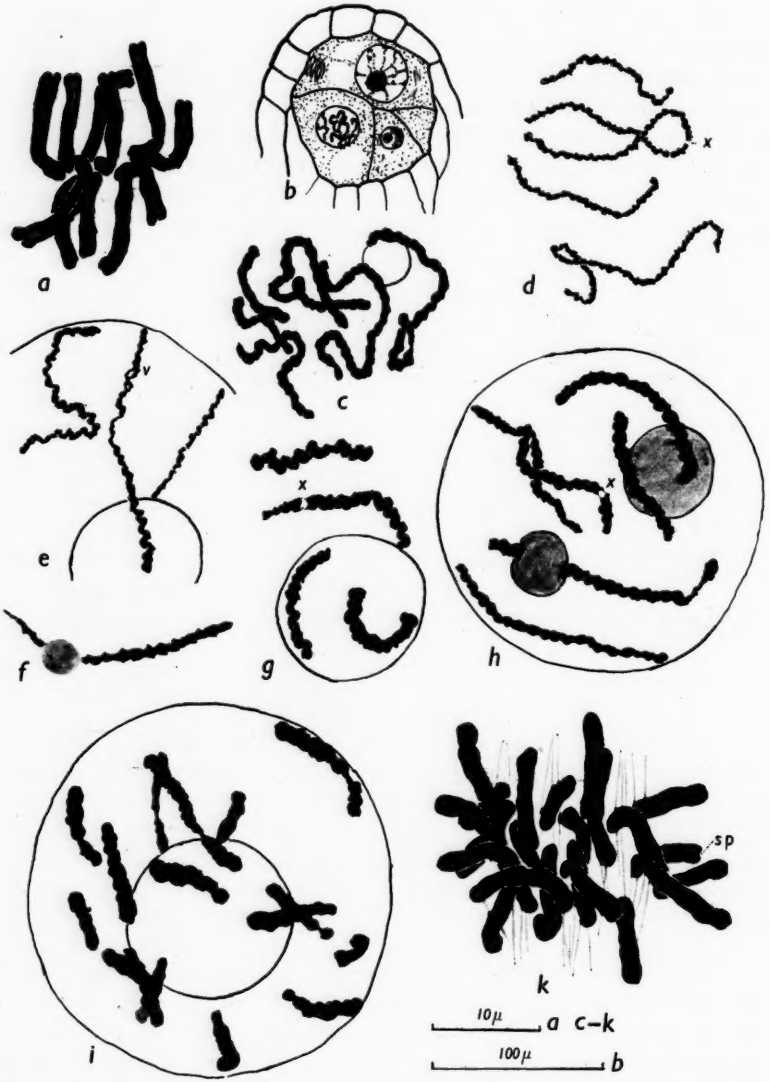

Fig. 2 *a*: Part of mitotic metaphase from integument-cell, untreated material, *b*—*k*: treated material, *b*: cell-complex from the mitotic division of E.M.C., one cell of which in prophase, in *c* under higher magnification, *d*—*i*: chromosomes from mitotic prophase-stages in the E.M.C. nucleus, *f*, *h*: nucleolar chromosomes, *k*: mitotic metaphase of the E.M.C. nucleus, 24 chromosomes, at *sp* a split chromosome.

should correspond to the heterotypic one is, I consider, entirely unjustifiable. As will be shown later, there follows upon this (1) a greater or less number of mitotic cell-divisions, (2) in many cases a true meiosis. The chromosomes are apparently single ones, although here and there are stages in which a longitudinal split quite clearly appears, even admitting that in many cases such a »longitudinal split» is only an optical illusion. In Fig. 2 *f* a nucleolar chromosome is illustrated with an extremely clear split, and likewise in Fig. 2 *e*. In Fig. 2 *k* one of the chromosomes to the right has a distinct split (*sp*). That a longitudinal division of the chromosomes actually takes place is, indeed, also shown by the often very lively production of cells with the diploid chromosome number in the E.M.C.

This very peculiar division of the embryo-sac mother cell occurs at a very early stage, when the nucleus is preparing for a meiotic division. Ovules with the E.M.C. nucleus in mitosis are found mixed among such where the E.M.C. nucleus is in leptotene or zygotene. It is clear that this stage is easily influenced by the drop in the temperature, a fact that STRAUB (1939) and other workers have already shown. As was mentioned above, a very small number of ovules situated at the top and bottom of the ovary are greatly retarded in their development and some of them seem to be abnormally formed. But the mitotic process described above is encountered in other portions of the ovary, including the top, central and basal portions. A couple of examples may be adduced. A newly opened flower that before fixation had been exposed to a considerable drop in temperature contained 259 examined ovules, where 102 (39 %) had the E.M.C. mitotically divided into a number of cells (hereafter denoted »div. E.M.C.»). The upper third of the ovary with 88 ovules contained 15 (17 %) div. E.M.C's, the central part, 89 ovules, 45 (50,5 %), and the basal part, 82 ovules, 42 (51,2 %).

At the close of the first mitosis a wall is formed between the two nuclei. Its direction differs much in different E.M.C's. Very often the wall is transverse or longitudinal, but it also and apparently most commonly runs obliquely through the E.M.C. The two resulting cells may divide again or not, in which former case a mostly well-defined group of cells arises from the E.M.C. Some different types are illustrated in Figs. 1, 3 and 4. If the first wall is transverse, and the cells continue to divide, the E.M.C. complex often assumes the appearance of a young embryo. The lower cell or cells assume the appearance of a chalazal foot-part, while the upper or the more micropylar cell develops in different ways. In rarer cases the division of the E.M.C. goes on so

long that a cell-complex of numerous mitotically formed cells entirely fill up the nucellus (Fig. 1 *g*). In Fig. 1 *h* this body of cells has been further differentiated into a chalazal portion with parietally oriented plasm and a large central vacuole, and in the upper half an assembly of plasm-rich cells that present the appearance of an archespore. The whole of this formation has however sprung from the E.M.C. through mitotic divisions.

Most commonly, however, at a far earlier stage, when only a small number of cells have been formed, a change occurs in the development of the cells. One or more cells, usually in the micropylar part of the cell-complex, pass over into a meiotic prophase. In other words, during preparation for meiosis and under the influence of cold the E.M.C. is first divided mitotically, since a pairing of the chromosomes is rendered more difficult, but chromatid formation can nevertheless take place. Subsequently, when the external conditions make it possible, cells formed in this way from the E.M.C. return to a meiotic direction of development. This process seems to me rather interesting, and in the subsequent development there hence arises in the nucellus varying and extremely peculiar cell-complexes, all issuing from the original E.M.C. Fig. 3 shows some of these types, which are all characterized by the fact that, after a number of cells have been formed by the mitotic method, some cell or other in the micropylar portion of the cell-complex passes over to meiosis. Fig. 3 *d*, *e*, *f*, *g* is from the ovaries that had been exposed to a powerful drop in temperature and thereupon allowed to develop in room temperature for 24 hours. The cell that in this way passes over into meiosis will therefore come to constitute a secondary E.M.C. Indeed, it actually develops as an E.M.C. In Fig. 3 *e* the nucleus is in pachytene, in *f* in heterotypic anaphase, somewhat obliquely cut. The primary E.M.C. has evidently divided mitotically with an oblique wall, the chalazal cell develops further mitotically, while the micropylar one becomes the E.M.C. At times a cell in the chalazal region may also pass over into meiosis as in Fig. 3 *d*.

Very often, however, it happens that after mitotic division all the cells in the E.M.C. complex assume a meiotic character, and the nucellus then apparently comes to enclose a »multicellular archespore». None the less, it is still a primary E.M.C. which, after a shorter or longer period of mitotic divisions, has entered into a new meiotic phase with secondary E.M.C. formation. Each of these can develop an embryo-sac in the usual way. The subsequent development, however, does not always take place synchronously. In Fig. 4 *c* a nucellus is illustrated

with three embryo-sacs; one of the large cells to the right has the nucleus in diplotene, the other to the left at the bottom is in metaphase I, and the third cell is two-nucleate. Fig. 4 *d* shows anaphase I in the right cell and anaphase II in the other. Fig. 4 *f* has two embryo-sacs springing from the primary E.M.C., the upper one in metaphase III with a haploid and a triploid nucleus; the chalazal embryo-sac is four-

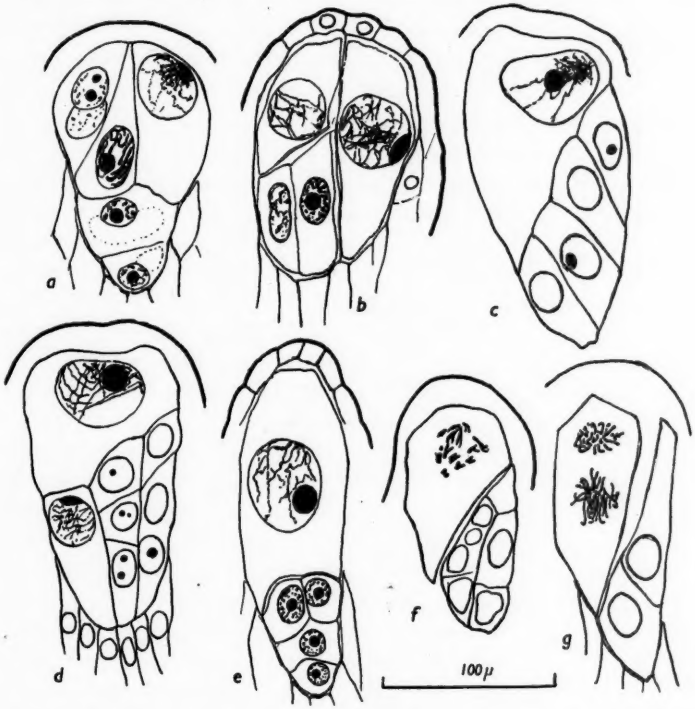

Fig. 3 *a*—*k*: Different configurations of derivates from the mitotic divisions of E.M.C.: apical cell or cells becoming secondary E.M.C., basal cell-groups forming a chalazal foot-part. In *d* one of the basal cells becoming secondary E.M.C., in *f* anaphase I and in *g* metaphase II of the secondary E.M.C., *c*—*g* from material which after cold treatment had remained in room temperature for 24 hours.

nucleated. Of a certain interest is the large cell in Fig. 4 *c*. The E.M.C. has here evidently divided with a longitudinal wall, the cell to the left has undertaken a new mitotic division, the daughter cells have become secondary E.M.C's. The cell to the right is much larger and may have begun a meiotic prophase but after that, through »internal reproduction»,

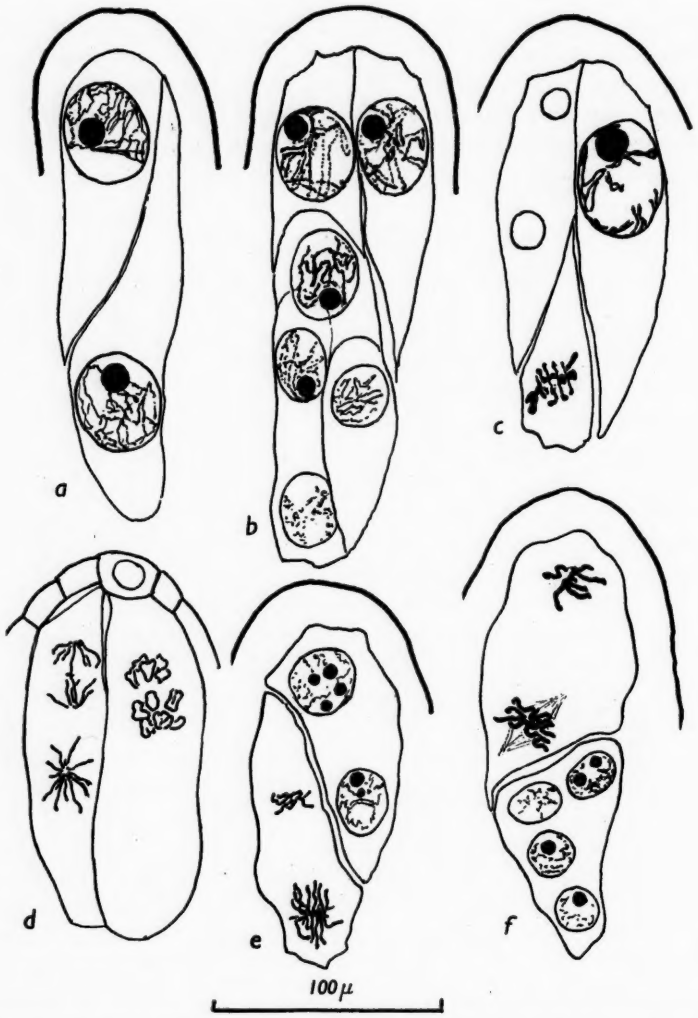

Fig. 4. Nucelli from cold-treated material which had afterwards been kept in room temperature for 24 hours. The primary E.M.C. has divided mitotically and all cells have developed into secondary E.M.C's (cf. the text).

passed over into a kind of restitution nucleus, which probably thereupon divides homotypically. Two clearer cases of this phenomenon will be submitted later.

An interesting circumstance with respect to this mitotic cell formation in the E.M.C. is that it seems to proceed so rapidly. Mixed up among the E.M.C's in the zygotene or the pachytene lie E.M.C's that have already divided into a greater or less number of mitotically formed cells. The cell-complexes in Fig. 1 *e* and *f* lie in the immediate vicinity of ovules with the E.M.C. nucleus in pachytene. DARLINGTON and THOMAS (1941) publish a case of supernumerary divisions of the vegetative nucleus in the pollen grains of a form of *Sorghum purpureo-sericeum*, where divisions apparently follow one another in rapid succession, »probably all within 24 hours».

As already pointed out, the experimental treatment of the material under review does not permit of an exact decision as to whether and to what degree the temperature had been decisive in respect of the deviations observed from the normal meiotic course. It is of course conceivable that it was not a question here of a cold effect, but that a genetically controlled character of this form of *Lilium longiflorum* may have caused the deviations in question. A number of circumstances seem to me to argue definitely against this latter assumption and to favour the cause being sought, instead, in the extreme fall in temperature that actually occurred. My material has been derived from the greenhouses of the same gardener for three years, and it has always been found that during autumn and spring the meiosis proceeded normally, while during the winter, under extremely low temperatures, the meiosis every year has shown deviations from the normal. The temperature in the greenhouse then sometimes fell to below + 5° C. A definite difference also appears with respect to the presence of these deviations in flowers on the same individual but of different ages. The younger the flower is, the higher is the percentage of E.M.C's that show mitotic divisions, evidently because a larger number of E.M.C's had the nucleus in very early meiotic prophase at the time of the cold shock. Table 1 below shows the number of E.M.C's with mitotically formed

TABLE 1.

	A		*B*		*C*		
Flower-length cm.	7,5	13,5	9,5	14	5,5	10	13,5
Number of analysed ovules...	103	217	182	119	304	262	227
Number of divided E.M.C's..	32	18	25	4	64	40	14
% of E.M.C's divided	31,1	8,2	19,2	3,4	21,0	15,3	6,2

cells in three different individuals *A*, *B*, *C*, with young and old flowers.

It therefore seems to me justifiable to assume that it is the action of cold during an evidently very sensitive stage that has caused the deviations in the development of the E.M.C. In *Gasteria* STRAUB (1939) has also found that just during leptotene the P.M.C. shows considerable deviations from the normal course of development, resulting in asynapsis as well as mitosis. What is surprising in *Lilium* is the active mitotic cell-formation in the E.M.C., which under favourable conditions may later retake a meiotic character.

A remarkable circumstance is that the meiotic phase sets in fairly simultaneously in adjacent ovules, independent of whether the E.M.C. is undivided or divided, i. e. independent of whether it is a question of a primary or secondary E.M.C. In *Lilium* synapsis (synizesis) in transition from zygotene to pachytene is characterized by the fact that the nucleolus »appears in the form of a cap» (FRANKEL, 1937). This phenomenon was described long ago in the literature under the term »Sichelstage» by LIDFORSS (1897) and others and is certainly an artefact. FRANKEL found »cap» nucleoli in the P.M.C's of two *Fritillaria* species, whereas in 24 other *F.* species the nucleoli were normal. DARLINGTON and LA COUR (1941) found »cap» nucleoli in both pollen- and embryo-sac mother cells in one *F.* species, but in five other species they could be found in the embryo-sac but not in the pollen mother cells. TISCHLER (1943) finds this variation of the nucleolus »ziemlich bedeutungslos». In a certain sense he is right: it is an artefact. On the other hand, it appears as though the nucleolar substance had assumed a softer consistency for a time, most likely at zygotene, and that under the influence of the fixing solution the nucleolus had been pressed against the nuclear membrane and taken a »cap» form. Usually, of course, at this stage the nucleus has only one nucleolus, but in *Lilium* two or three »cap» nucleoli are sometimes encountered. When the nucleus thereupon passes over to pachytene, the nucleolus at fixation acquires a rounded form and a free and central position in the nucleus. In normal cases the »cap» form lasts merely a short time, but in cold-affected *Lilium* material this stage went on for a longer period, judging from its frequent occurrence. As is known, under the influence of cold the course of meiosis is more or less retarded. In any case, the »cap» formation indicates that the nature of the nuclear substance undergoes some alteration during zygotene that brings about a characteristic reaction to the fixing medium at a definite point of time. The differ-

ence found by DARLINGTON and LA COUR in the respect mentioned in P.M.C's and E.M.C's is very interesting.

DEFECTIVE WALL FORMATION IN THE EMBRYO-SAC MOTHER CELL.

Besides the above-mentioned deviations from the normal course of development in the ovule of treated *Lilium* material there occurs, although more rarely, a type that might be briefly described as a defective wall-formation in the E.M.C. without nucleus division. Fig. 5 *a*

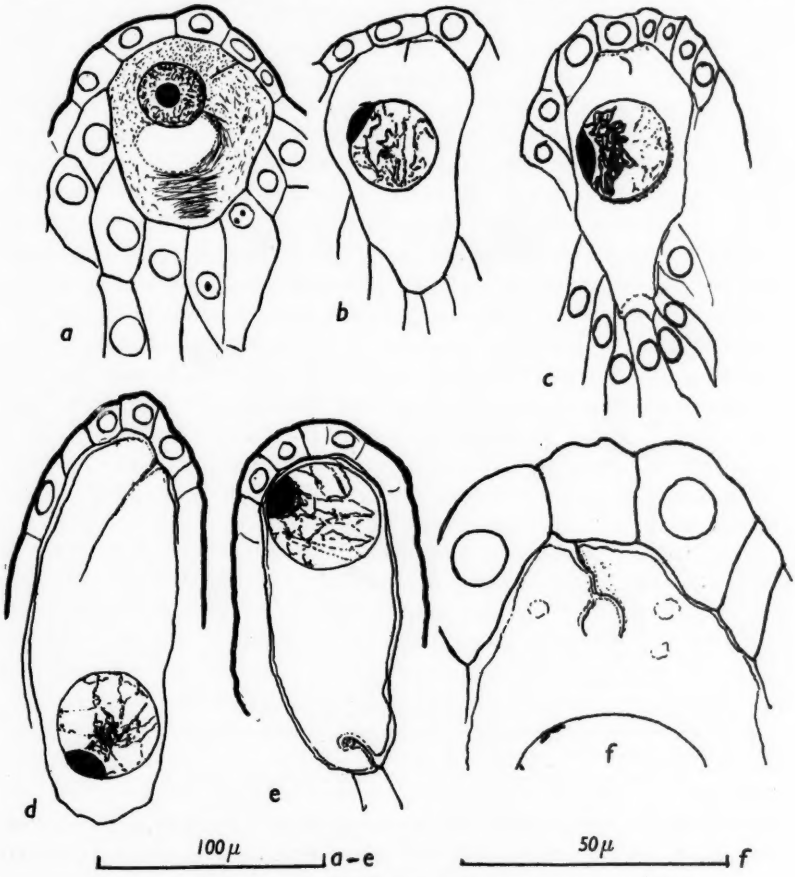

Fig. 5. Different types of defective wall-formation in the E.M.C.

illustrates this. The E.M.C. nucleus is at the end of a resting stage, and, as is usual in treated material, there is a thready structure as well as a

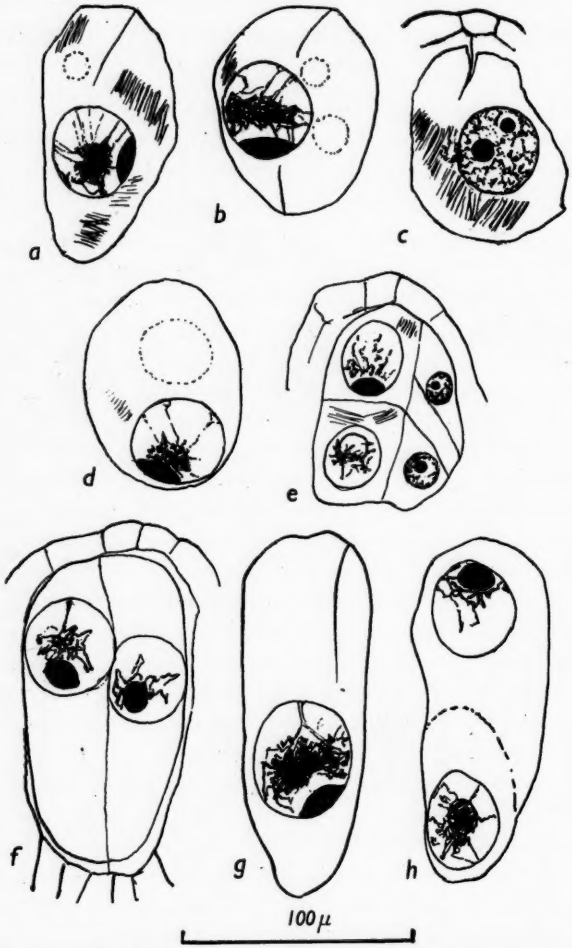

Fig. 6. Defective wall-formation. *a*—*e*: E.M.C. from five adjacent ovules, in *e* a cell-complex mitotically developed from the E.M.C., *f*—*h*: E.M.C. from three ovules on the same section, two E.M.C's two-celled, *g* with uncompleted wall.

vacuole in the cytoplasm. In the upper part of the cell, close to the epidermis, a small wall-fragment is seen that clearly issues from the inner wall of the epidermis (Fig. 5 *d*, *f*; Fig. 6 *c*). The wall may extend

a shorter or longer distance into the E.M.C. Usually it springs from the nucellar epidermis, though now and then it has its origin in the basal part of the E.M.C. (Fig. 5 *e*). It is generally very short (Fig. 5 *b*), but sometimes extends far into the E.M.C. and even along its entire long side. The whole gives the impression of a commencing division of the E.M.C. as in the cases referred to above, though the process has subsequently stopped while the nucleus still remains seemingly undivided. This uncompleted cell wall-formation will hereafter be denoted as *W*. Ovules and E.M.C's of this type occur intermingled with divided or undivided E.M.C's. Fig. 6 *a—e* illustrates five E.M.C's from five ovules situated close together, three of which have *W*. Fig. 6 *f—h* shows three E.M.C's from three ovules in the same section. Two are divided with respectively a longitudinal and an oblique wall and are binucleate, the central cell having a distinct *W* and a large nucleus with »cap» nucleolus. The frequency of this phenomenon is rather variable. In one bud, 5,5 cm. in length, 5,3 % of the ovules showed E.M.C's with distinct *W* occurrence, in another, 8,5 cm. in length, the corresponding figure was 4,4 %. Only in two almost fully open flowers was this formation entirely absent, while in the same flowers about 18 % of the ovules had mitotically divided E.M.C's. In material from autumn and spring, i. e. under more normal conditions, a lamel of this nature was extremely rarely found. This formation is not at all to be compared with the short-lived wall-like structures occurring during meiosis and often described in *Lilium* for interkinesis stages. What significance it may have is not easy to determine. *In all cases in which there are division stages in conjunction with W the chromosome number has been found to be doubled.* It might therefore be supposed that a wall-fragment of this kind stands in some connexion with chromosome division without nuclear division, a kind of endomitosis or double reproduction. As was previously stated, not so small a percentage of the E.M.C's has undergone one or two mitotic, instead of meiotic, divisions, and it is therefore conceivable that such a mitosis may now and then have been arrested in prophase and, in connexion with the weak spindle formation, continued as inner reproduction. A couple of examples will be given below that possibly argue in favour of such an interpretation.

Fig. 7 *a* and *c* illustrate part of anaphase I in E.M.C's from two different individuals. A control count gave the result that each chromosome group consisted of about 24 split chromosomes from 24 bivalents. Fig. 7 *b* shows a metaphase I of somewhat irregular appear-

ance owing to the occurrence of both bivalents and univalents. In this case, presumably, the pairing between certain chromosomes was dis-

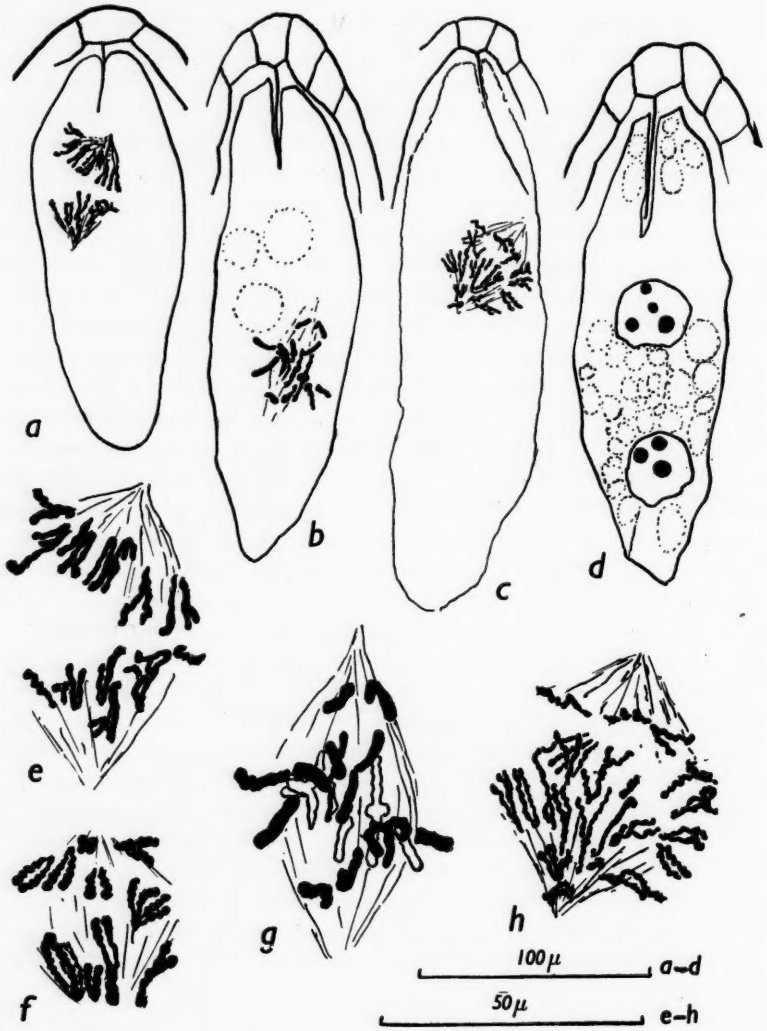

Fig. 7. Diploid heterotypic division in primary E.M.C. with defective wall-formation, *e*, *g*, *h* the nuclei of *a*, *b* and *c* under higher magnification, *f* anaphase from untreated material.

turbed during an earlier period under the influence of cold. Some bivalents are nevertheless to be distinctly seen among the univalents. Here, too, the chromosome number was doubled. All three examples exhibit the characteristic wall-formation very clearly in the upper part of the E. M. C's. In all three cases the E.M.C. in the other adjacent ovules was in the 4-nucleus stage. The material is derived from individuals which after a cold period had developed further under room temperature for a couple of hours and 24 hours respectively. Fig. 7 *d* represents an interkinesis stage and probably constitutes a later stage of the anaphase pictured in *a* and *c*. The adjacent ovules had embryo-sacs in the 4- or 8-nucleate stage. Of some interest is the fact that such stages of division with doubled chromosome number and *W* occurrence evidently *represent a retarded development.*

Further illumination of the supposed connexion of this peculiar wall-formation with chromosome doubling is given by Fig. 8 *a*. The E.M.C. nucleus is in prophase, which seems however to represent an intermediate stage between mitosis and asynaptic prophase. Although the total number of chromosomes cannot be exactly stated, as the nucleus falls in two sections, it is certainly about 48. At *W* a distinct wall is seen to project a bit into the cell, springing from a chalazal cell. A chromosome doubling has occurred, but to what extent this has its cause in inner reproduction is difficult to decide. A more pronounced paired arrangement of the chromosomes would have been expected. The adjacent ovules had the E.M.C. nucleus in pachytene.

A rather similar condition, though at a later stage of development of the E.M.C., is shown in Fig. 8 *b*. The E.M.C. has divided mitotically into two cells, and the chalazal one has commenced a further division. This is at metaphase, and in normal cases the subsequent course would be as indicated in Fig. 8 *e*. Here, however, a chromosome doubling has also taken place in connexion with the formation of a weak, scarcely visible cell-wall (*W*). The chromosome number is 48 (Fig. 8 *c*, *d*).

A couple of examples adduced above seem to support the assumption that in cases where the chromosome number has admitted of being determined in an E.M.C. with a defective wall a chromosome doubling has occurred. To draw from this the conclusion that the wall-fragment is, as it were, an indicator of the occurrence of endomitosis is perhaps not unjustifiable or improbable, although objections to this may be urged. Several observers have already shown that at endomitosis an increase in volume takes place. From their investigations of double chromosome reproduction in *Spinacia* GENTCHEFF and GUSTAFSSON

(1939) have come to the conclusion that »the pairing at metaphase and prophase cannot arise in any other way than by repeated chromosome reproduction before the visible prophase stages». In the *Lilium* material chromatin occurs in distinctly larger quantities in the *W* cells than in the other ones (cf. Fig. 6). This is, of course, a rather indefinite subjective measure, and a determination of the nuclear volume ought

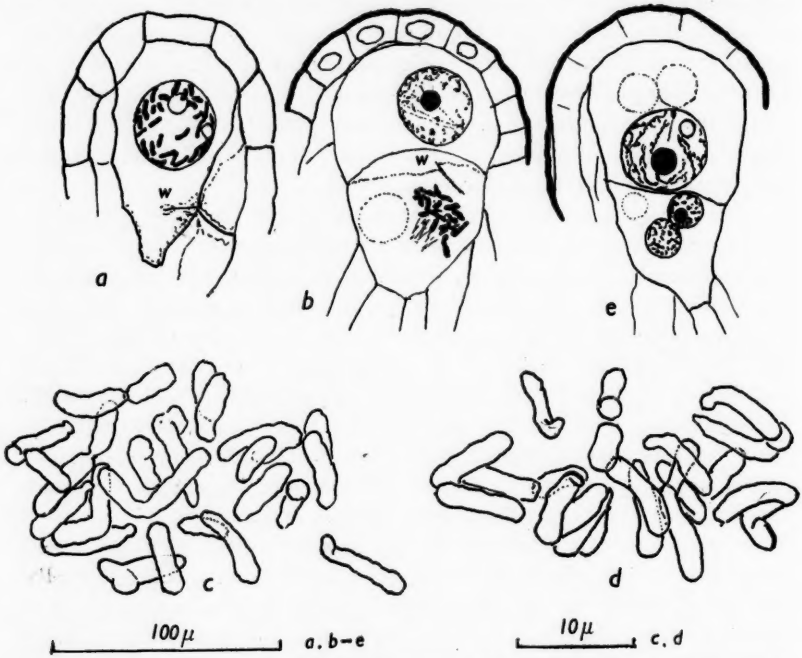

Fig. 8. *a*: Prophase in E.M.C. with defective wall-formation, part of the nucleus, which had about 48 chromosomes, *b*: metaphase with 48 chromosomes in a basal cell from the mitotic division of the E.M.C. The cell has an uncompleted wall at *w*; *c*, *d*: the nucleus in *b* under higher magnification. *e*: analogous stage to *b* without *W*, the lower cell has fulfilled a diploid mitosis.

to give more reliable information. However, a comparison of the nuclear size has not revealed any noticeable or tolerably constant difference in *Lilium*. In some cases the nucleus was certainly distinctly larger in the *W* cells than in the normal ones, but the size of the nucleus is as a general rule rather variable during the pachytene stage.

From the preceding review it thus seems evident *that in all the cases in which defective wall-formation in the E.M.C. has been found*

and in which the chromosome number could at the same time be determined, a chromosome doubling has taken place. On the other hand, the examination of a rather extensive cold-affected *Lilium* material has shown that meiotic stages with 24 bivalents instead of twelve have occurred *only* in conjunction with *W* formation. Now, if the assumption made above of a connexion between endomitosis and *W* formation is correct, then this should imply that the doubling of the chromosomes must have taken place before the meiotic prophase started, as otherwise the diploid number of bivalents cannot be conceived to arise. This does not however exclude the possibility that an inner reproduction in a pre-meiotic stage can give rise to a doubling of the bivalents even without wall-formation. In my material, however, I have not encountered anything of this kind. The final result of the development of this E.M.C. type will thus be the origin of diploid embryo-sacs.

OTHER CASES OF CHROMOSOME DOUBLING IN THE EMBRYO-SAC MOTHER CELL OF LILIUM.

That under the influence of the extreme temperature a chromosome doubling nevertheless takes place in many cases without *W* formation has been demonstrated by several research-workers. Indeed, STRAUB has even elaborated a method designed to give the greatest possible production of diploid pollen cells and egg cells by cold treatment. I have also found several such cases in my *Lilium* material. But in all these cases it is not a question of an increase in the number of *bivalents* during meiosis, but rather the development of a form of restitution nucleus in conjunction with asynapsis. The fact is that at the first division of the E.M.C. nucleus the chromosomes often have the same shape as during interkinesis. In other words, if the E.M.C. nucleus has already begun meiotic prophase, the low temperature will render chromosome pairing more difficult and the pre-requisite condition is thus at hand for transition to interkinesis through formation of a restitution nucleus or for direct transition to a homotypic division with a diploid number of split chromosomes.

Fig. 9 *a*, *b* shows such a case. The nucleus of the E.M.C. is in metaphase. The spindle-figure is well developed, in contrast to what is usually the case in treated material. Perhaps the reason for this is that after the cold treatment the individual was kept five hours in room temperature before fixing was undertaken. The figure shows only part of the chromosomes. These were 24 in number and had the appearance

characteristic of the homotypic division. At the top of *b*, to the right, a chromosome is seen with a median centromere. The chromosomes are considerably shorter and more contracted than is usual in the second division of *Lilium*, and resemble the corresponding chromosome stage in *Hieracium* (ROSENBERG, 1927). The cold shock has presumably struck the nucleus during early zygotene, the chromosome pairing has been disturbed, a kind of asynapsis has followed and concurrently the

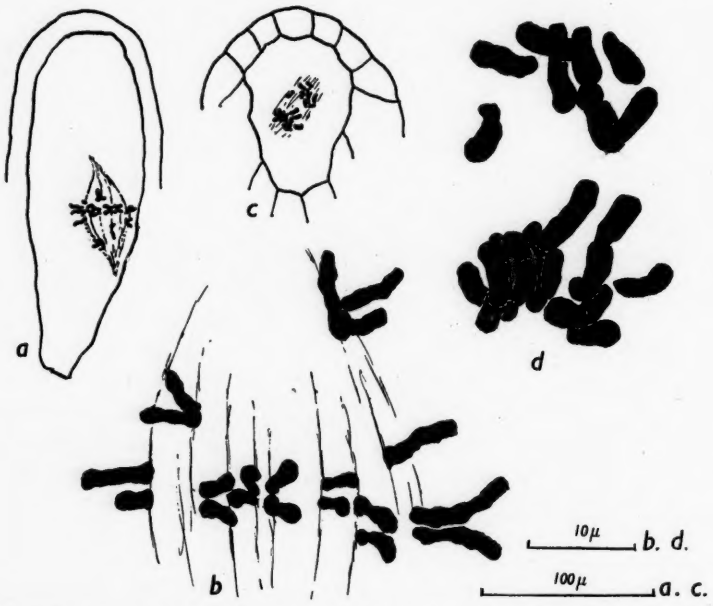

Fig. 9. Formation of restitution-nucleus: *a*, *b*: embryo-sac mother cell in older stage with nucleus in metaphase, 24 chromosomes (only a part of them pictured) of homotypic appearance, *b*: under higher magnification, *c*, *d*: very young E.M.C. in asynaptic anaphase, probably developing to a restitution nucleus (cf. the text).

chromatids have commenced to separate, though they remain attached to the undivided centromere for a considerable time.

In Fig. 9 *c*, *d* an anaphase is illustrated from a very early stage in the development of the E.M.C. The adjacent ovules had the E.M.C. nucleus in the premeiotic stage, somewhat like the E.M.C. nuclei pictured in Fig. 1. The stage is very similar to a »semiheterotypic» anaphase with considerably contracted chromosomes. In my opinion it may be conceived as representing an earlier meiotic phase of such a nucleus

as is illustrated in Fig. 9 *a*. It is easy to imagine that the subsequent course of Fig. 9 *c* corresponds to a restitution-nucleus formation (taken in its widest sense) and will consequently be followed by a metaphase with interkinesis chromosomes, like Fig. 9 *a*. Fig. 10 *d*, *e* shows nuclei from an ovary with an E.M.C. divided mitotically into two cells, with a longitudinal wall. Each cell in itself constitutes a secondary E.M.C. in the uninucleate stage. One of the cells has the nucleus in prophase with 24 split chromosomes, some of which are illustrated in Fig. 10 *d*. It is at once clear that these are interkinesis chromosomes, though in the uninucleate stage of the E.M.C. At *x* there is evidently one of the large chromosomes with median constriction. The following metaphase would probably present an appearance indicated in Fig. 9 *a*, though somewhat more regular. The result will be two diploid nuclei. An examination of a large number of adjacent ovules discloses the rather striking fact that among ovules with haploid 4-nucleate stages there often occur one or two with binucleate E.M.C's, although the nuclei of these are considerably larger than those found in normal interkinesis and in the »resting» stage, i. e. are without split chromosomes characteristic of interkinesis nuclei. This mode of origin for diploid nuclei during meiosis resembles in the main the types found by STRAUB in *Gasteria*. To quite a different type belongs the previously described form combined with *diploid bivalent formation* and defective wall-formation. In all such cases, however, it is a question of the first meiotic division of the primary E.M.C.

As was mentioned above, the secondary E.M.C's, arising after mitosis, usually developed later in a normal manner into haploid embryo-sacs. But here, too, there may sometimes occur a development similar to that just described in Fig. 9. Fig. 10 *a*—*c* illustrates such a case. The primary E.M.C. nucleus has divided mitotically with a longitudinal wall into two cells. In both of these a meiosis has started but has followed a normal course to the 4-nucleate stage only in one of them (the cell lying behind to the left). In the second, front cell the nucleus is in metaphase, though not a heterotypic one. The condition can be explained thus, that this cell had commenced a meiosis at the same time as metaphase I of the second cell, but under the influence of the low temperature chromosome pairing was inhibited and a spindle-figure developed, instead, with more or less regularly arranged »interkinesis» chromosomes, thus in essentials as in formation of a restitution nucleus. It will be seen in Fig. 10 *b*, *c* that the 24 chromosomes have the appearance characteristic of interkinesis, even if they are shorter,

more contracted than in a normal *Lilium* interkinesis. The chromosomes are clearly divided, with spread out chromatids and as yet undivided centromere. An interesting feature is that one of the cells has already reached the 4-nucleate stage, while the other, which so to say has jumped over the first division, has got behindhand in its development, i. e. the uninucleate cell has a spindle-figure with the appear-

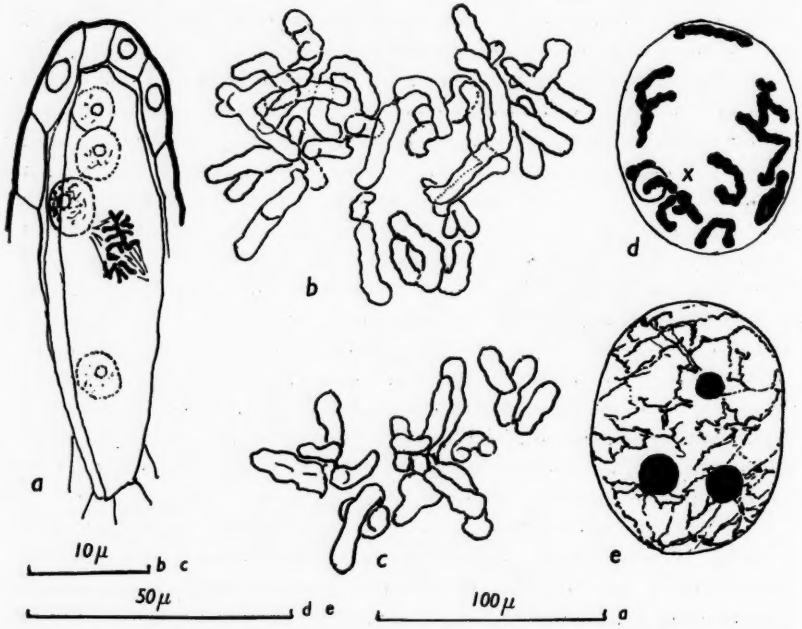

Fig. 10 *a*: E.M.C. divided in two cells after mitosis; one has undergone a normal meiosis and is now 4-nucleate. The other cell has its nucleus in metaphase after formation of restitution nucleus, 24 chromosomes, in *b* and *c* under higher magnification. *d* and *e* show nuclei from a similar but earlier stage of development, the chromosomes in *d* being as at interkinesis.

ance of a homotypic metaphase while the second, haploid, one has already completed this division. The adjoining ovaries, which had developed more or less normally, had the primary E.M.C. in the 4- or early 8-nucleate stage. The fact that here and there an ovule with retarded development is encountered among the otherwise normally developed ovules with their E.M.C's in the 4-nucleate stage receives its satisfactory explanation in this way.

Some further interesting deviations from the normal scheme have also occurred as a result of the low temperature, but they will only be briefly mentioned here. A more detailed account and discussion of these would require more extensive material treated by more exact methods.

In some cases rather far advanced uninucleate E.M.C's in metaphase were met with, but they had long, split chromosomes, the exact number of which it was difficult to determine, though it was probably diploid. They doubtless indicate a development of the E.M.C. in a purely mitotic direction. In more advanced ovaries, affected by the low temperature, developmental stages of the embryo-sacs were found that can illustrate the subsequent course of the different types treated in the foregoing pages. In the normal, haploid embryo-sac at the 4-nucleate stage the fusion of the three chalazal nuclei into a triploid nucleus may rather often be absent and, instead, all four nuclei divide again separately and give rise to an 8-nucleate embryo-sac. An embryo-sac of this type thus deviates genetically from the *Lilium* type in that the endosperm nucleus, after fusion of the polar nuclei, will be diploid instead of tetraploid. To what extent this condition is connected with the fall in temperature is difficult to decide. DARLINGTON and LA COUR (1941) have pointed out something very similar in an account of the genetics of embryo-sac development.

It seems obvious, on the other hand, that a temperature effect is present in those cases where the course of meiosis appears to be fairly normal but where the chiasma frequency is weak and the bivalent chromosomes lie distinctly separate at diakinesis although close to one another. At metaphase I, besides bivalents, also univalents are encountered.

A remarkable condition, finally, is the difference in size of the E.M.C. during corresponding stages in normal grown material and material grown under low temperature. The growth of the embryo-sac mother cell seems able to proceed fairly unhindered in the cold-affected material, whereas the process of division in the nucleus has been greatly retarded. Connected with this is the condition referred to above that »cap» nucleoli are found in the E.M.C. considerably more frequently in treated than in untreated material. A comparison is given below of the lengths of the embryo-sac mother cells during different stages of meiosis in normal and in cold-affected material.

	Pachytene	Metaphase	4-nucleate stages
Untreated	120 μ	126 μ	129 μ
Treated	145 μ	166 μ	173 μ

Finally, it may be mentioned that a prophase in a secondary E.M.C. has been observed, although very rarely, with 48 extremely contracted and for the most part rounded chromosomes, reminiscent of a »semiheterotypic» prophase in *Hieracium levigatum* (ROSENBERG, 1927, Fig. 3 *A*, *B*). STRAUB has described such a case in *Gasteria*, »kurze, dicke Chromosomen» after cold shock.

DISCUSSION.

GUSTAFSSON (1939, 1942) and STEBBINS (1941) have pointed out that the study of meiotic abnormalities found in apomicts is one of the most promising methods for an attack on the problem of the mechanism of meiosis. SAX and SAX (1935), SAX (1937) and STRAUB (1939) as well as others have further shown that variations in temperature have an effect on nucleus and cell-division that can illuminate the same problems very considerably. It is true that the investigations on *Lilium* described in the foregoing pages are not based on a material that can be considered as entirely satisfactory in the light of exact experimental demands. Nevertheless they have given results that are of significance for the interpretation of the general influence low temperature has on the course of meiosis and by that indirectly on its nature.

What is then remarkable in the first place is the mitotic nucleus and cell-division found in a rather high percentage of the cases examined during the earliest phase of E.M.C. development. Even at an early stage the embryo-sac mother cell in *Lilium* is sharply separated from surrounding cells in the nucellus, so that no doubt whatever can arise as to its delimitation and later development. In a rather substantial percentage of the examined cases there appear two or more E.M.C's in the nucellus, and sometimes even a large number. The occurrence of double E.M.C's in *Lilium* has been previously reported, though in such cases with nucellar tissue between them. DARLINGTON and LA COUR (1941) have found »twin mother cells» even without such tissue in *Fritillaria*, without making any statement as to their mode of origin. The deviations from the normal course found by me in *Lilium*, however, are of an essentially different character. The »multicellular archespore» has here quite clearly arisen within the E.M.C. by means

of mitotic divisions beginning very early. These divisions have not occurred in connexion with an early meiotic pairing of the chromosomes. They consist rather in a lively and rapid process of mitosis in the E.M.C. This results in the formation of a cell-complex which always keeps the general form of delimitation of the E.M.C., especially towards the chalaza. During the whole of this time the nuclear divisions are *mitotic* in character, which is evident among other things from a study of the prophase at the repeated division as well as from the finally occurring meiosis, where the chromosomes form 12 pairs at metaphase. If the mode of origin were unknown, this tissue would certainly be described as a »multicellular archespore». But the sporogenous complex is of secondary origin, having arisen from a primary E.M.C. after mitosis. With the very greatest probability it is the low temperature which has retarded the meiosis and in many cases caused the first division of the E.M.C. nucleus to take the mitotic direction, which is less influenced by a fall in temperature. This division has taken place at a very early stage, before the point when meiosis would normally commence. In other words, the division of the nucleus is directed in a mitotic instead of a meiotic development.

Analogous conditions have been demonstrated by GUSTAFSSON (1942) in *Hieracium* at the development of the P.M.C's. In a couple of works (ROSENBERG, 1917, 1927) I have shown that in the P.M.C's of different species of *Hieracium* different types of division could be distinguished and also that these types could be found within the same species. In certain cases, especially in rather old anthers, the division could even proceed in a more or less typical meiotic direction. GUSTAFSSON (1939, 1942) has made the important observation that the different »types» stand in some connexion with, among other things, the *time* for the commencement of the division in the P.M.C. On the basis of a rich material he has been able to formulate the principle that the earlier a division starts in the P.M.C. the more mitotic will its course be. The mitotic division of the E.M.C. nucleus now shown in *Lilium* is doubtless an analogous phenomenon. Chromosome pairing has become retarded during zygotene through a drop in temperature, which does not however prevent the initiation of a mitosis. This seems to me to show that in the E.M.C. of *Lilium* the *chromosomes at the immediately preceding telophase are not constituted differently to what they are at the earlier nuclear divisions*. We must not perhaps build overmuch on the reports of split chromosomes in leptotene; they may be due to erroneous optical judgment in some cases. But in the case pictured by me in Fig. 2 *d*

the split was quite specially distinct. Certainly it is a question of a mitotic prophase in the E.M.C., but at a very early stage. Moreover, if DARLINGTON's precocity theory is taken as a starting-point, it would surely be expected that when, as here, chromosome pairing is inhibited by a fall in temperature the consequence will be that the division of the nucleus gets retarded. Instead, it is initiated at an especially early stage, though in a mitotic direction.

Admittedly it is remarkable that a mitosis can proceed in an E.M.C. under low temperature, even if it is taken into account that mitosis is disturbed less by a fall in temperature than meiosis. The usual effect is, of course, that especially the metaphase stage is inhibited (BARBER and CALLAN, 1942). Unfortunately, I cannot submit any exact data as to the degree of cold, but it is of course conceivable that a point occurred when meiosis was prevented but mitosis could still take place. For the rest, the conditions here become rather peculiar owing to the omitted chromosome pairing. The E.M.C. is a very large cell and, as the nuclear division goes in a mitotic direction, it is conceivable that a repeated nuclear division will ensue. In the literature there are reports of continued mitotic division in the archespore even when meiosis is inhibited. From an experimental investigation of excised anthers in *Lilium longiflorum* GREGORY has obtained results which are to some extent analogous. Anthers at different stages of development were grown in vitro for several days. It was found that sporogenous cells in anthers at 3 mm. in length continued mitosis for twelve days. There is admittedly a difference between the sporogenous tissue in anthers and the archespore in the nucellus. In anthers the archespore can divide repeatedly after it has been formed, also under normal conditions. In the nucellus the E.M.C. is a subepidermal cell that in normal cases has a long resting-stage and does not divide until meiosis. The continued mitotic division in GREGORY's experiment is considered by him to depend on the anthers having been shut off from the supply of certain substances conducive to meiosis, i. e. to some extent a correspondence to the suppressed meiosis in the E.M.C. of treated *Lilium* ovules.

The described deviations from the normal development might perhaps give rise to doubt as to whether the drop in temperature is actually the cause. That we have to calculate here with an action coming from without appears to me to be clear from the comparison given on page 73 of the occurrence of »div. E.M.C's» in younger and older flowers from the same individual. A comparison between the occurrence of »div. E.M.C's» in treated and untreated plants also points

to a fall in temperature being the operating factor. Further support for this conclusion was given by a close study of the distribution of »div. E.M.C's» in the ovary of various old flowers on the same individual. The appended table gives the distribution of ovules with »div. E.M.C's» in the upper third, the central and the lower portion of the ovary in three flowers, *A*, *B* and *C*, of different length and age from the same plant:

Length of flower	*A*: 5,5 cm			*B*: 10 cm			*C*: 13,5 cm		
	No. of ovules	Div. E.M.C's	% div. E.M.C's	No. of ovules	Div. E.M. C's	% div. E.M.C's	No. of ovules	Div. E.M.C's	% div. E.M.C's
Upper part...	107	6	5,6	86	3	3,5	80	0	0
Central part...	106	23	21,7	87	11	12,6	75	4	5,3
Basal part ...	91	35	38,5	89	26	29,2	72	10	13,9
	304	64	21,0	262	40	15,3	227	14	6,2

From this it is seen that in the youngest flower, *A*, »div. E.M.C's» occurred fairly evenly distributed in the two lower thirds of the ovary, whereas the upper third had rather few. In flower *B* the percentage of »div. E.M.C's» is substantially lower in the central part also, but still rather high in the basal part. In flower *C* »div. E.M.C's» were entirely absent in the upper third, were very few in the central portion and relatively few in the basal portion, about as in the central portion of *B*. I cannot give any data as to the degrees of cold or the time the flowers had been exposed to a drop in temperature, but merely state that there had been a specially cold period, which had also made itself felt in the greenhouse. A probable explanation of this distribution is presumably that during an earlier phase, when *C* was in the bud stage, the temperature had been more normal and had thus not induced »div. E.M.C's» in the ovules, which were then in the pre-meiotic stage. Subsequently, a drop in temperature occurred. This would account for the number of »div. E.M.C's» in the basal part of *C* and central and basal parts of *A* and *B*, since development proceeds basipetally and hence ovules with the E.M.C. nucleus in a stage sensitive to temperature-drops are more probably present.

In the group of cells, formed as a result of the mitotic division of

the primary E.M.C., a complete or partial transition occurs from somatic cells to secondary E.M.C's, a rather interesting circumstance. It is then mostly the micropylar cells that re-assume sporogenous character. The external conditions, a more favourable temperature, evidently played here a conducive part. There seems to be little room for assuming that the last pre-meiotic division in this cell-complex should differ from other mitoses (compare the precocity theory). Especially a case such as in Fig. 3 *d* is rather illuminating. One cell in the »foot-part» to the left has evidently also become sporogenous, the nucleus having a »cap» nucleolus. It may be said that the vegetative phase is altered in a generative direction as in the normal development of the E.M.C. Various theories have been advanced to explain such a change, and several workers have suggested that the change is effected by special meiosis-producing substances or hormones. The case just adduced is possibly interpretable in this direction. The results from GREGORY's experiments cited above can also, of course, be subject to a similar explanation. He writes on page 691: »The possibility of some accessory substance necessary for the normal sequence of events prior to diplotene in meiosis is more possible».

Another interesting point in this *Lilium* material is the defective wall-formation in the E.M.C. and its connexion with a doubling of the chromosome number. Otherwise endomitosis or inner reproduction usually occurs unconnected with a wall-formation of this kind. I must also admit that the conclusion that defective wall-formation is to be interpreted as an indication of a previous endomitosis is rather uncertain. The chief argument that can be advanced in its favour is, as already mentioned, the fact that in all the cases in which the chromosomes could be counted in connexion with defective wall-formation chromosome doubling was found to have taken place. In the synapsis and pachytene stage cell-nuclei from such cells generally appear to be considerably richer in chromatin substance than the others. Here, however, it is a question of a very sudden and rapidly proceeding cell-formation in a relatively large cell. Ovules with divided and undivided E.M.C's lie mixed up among one another and all in the same stage of development. SAX (1937) presents a case having an analogous wall-formation in the pollen-cells of *Tradescantia*. It seems to me, however, wiser to defer the discussion of this question until a more extensive material can be analysed.

SUMMARY.

(1) Plants of *Lilium* subjected to low temperatures have been examined with regard to the development of embryo-sac mother cells and the course of meiosis.

(2) As a result of the fall in temperature the pairing of the chromosomes is retarded and in often a rather high percentage of cases the E.M.C. nucleus shows a premature onset of prophase in a mitotic direction.

(3) Through such a mitotic division there issues from the E.M.C. a cell-complex composed of a greater or less number of cells, all or some of which later, under favourable temperature conditions, develop into secondary E.M.C's.

(4) In some E.M.C's a defective wall-formation appears during the pre-meiotic stage. This is interpreted as the result of an endomitosis that has taken place within the E.M.C. nucleus, for in all such cases in which it has been possible to count the chromosomes a doubling of their number has been registered.

(5) The occurrence of metaphase I with the diploid number of bivalents has proved to be co-ordinated with defective wall-formation.

(6) Diploid embryo-sacs can also appear through a formation of restitution-nuclei at a more or less early stage in the course of meiosis.

(7) The significance of the observations made for the illumination of the question of the relationship between mitosis and meiosis is briefly discussed.

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ABORTIVE EGG CELLS IN THE MOUSE

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THE author has studied the maturation divisions of the egg cells in the ovaries of the mouse. In the investigations it has been observed that the maturation divisions take place in two principal ways, one of which leads to the development of normal egg cells and the other to so-called abortive egg cells being produced. The anaphase and telophase of the first maturation division seem to be the stage that determines whether the egg cell develops into a normal haploid gamete or into an atypical polyploid egg cell.

In normal telophase the middle of the spindle contains a beltlike constriction, which is the spot at which the plasma later on constricts itself and forms the first polar body (Fig. 1). The telophase is followed by a short interkinesis. This interkinesis leads to the formation of the metaphase plate of the second maturation division. The anaphase and telophase of the second maturation division do not occur until after the fertilization of the egg cell. The chromosomes often form fused fibres of chromatin in the polar body produced in the first maturation division. Only seldom do the chromosomes form a regular second metaphase plate. If such is produced, it is also followed by the division of the polar body in two. Thus, in some cases there may be two polar bodies on the surface of the egg cell, which nevertheless does not indicate that the second maturation division has occurred.

The chromosome conditions differing from the normal are due to the fact that no polar body at all is produced in the telophase of the first maturation division (Fig. 2). Compared with the corresponding normal stage the only difference is that the spindle lies deep under the surface of the egg cell. In addition, the spindle lies more or less tangentially. In normal cases the spindle lies in the direction of the radius of the egg cell and near its surface. A consequence of the abnormal position of the spindle is that all the chromosomes stay in the egg cell during the interkinesis following the telophase, and no polar body is produced at all (Fig. 3). Such an interkinesis can lead to cases in which two metaphase plates of the second maturation division develop in the same egg cell, without any polar body developing or

without the egg cell even otherwise dividing into two parts. Or the oocyte may also divide into two equal halves, which is caused by an extraordinarily strong constriction of the plasma. It happens very seldom (one case out of the 1548 I examined) that the chromosomes form one regular metaphase plate of the second maturation division (Figs. 4 and 5). *In this case the oocyte has developed into a diploid cell instead of a haploid one. At the same time it proves that a polar body mechanism differing from the normal can cause polyploidy.* In most cases, however, something quite different occurs. The development seems to stop at the interkinesis, and during it phenomena occur that give the egg cell **an atypical and polyploid character.**

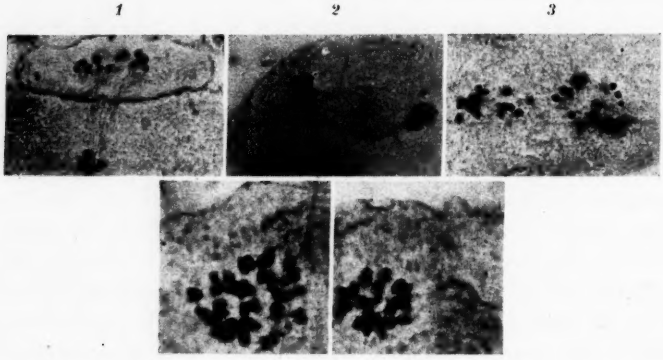

Fig. 1. A normal telophase of the first maturation division. The first polar body has developed. The polar body is constricted off at the beltlike ring in the middle of the spindle. (× 800.) — Fig. 2. A telophase differing from the normal of the first maturation division. No polar body is developed, as the spindle lies tangentially. (× 800.) — Fig. 3. The interkinesis following an abnormal telophase. All the chromosomes remain in the egg cell, as no polar body has developed. Notice the differences in size of the chromosomes. (× 800.) — Fig. 4 *a*. A diploid metaphase plate of the second maturation division, which has so developed that in the interkinesis following the abnormal telophase all the halves of the bivalents have formed one metaphase plate. All the chromosomes were not in the same section, but, as the figure shows, part of the chromosomes were in another section. (× 1600.) — Fig. 4 *b* shows what the haploid metaphase plate of the second maturation division looks like. Above, to the right, the polar body. (× 1600.)

When I commenced my researches, my attention was first of all attracted by a stage of division resembling a late diakinesis, in which the number of the chromosomes varied to a noticeable degree. There were, for instance, cases in which the chromosomes were fewer than

the haploid number and others in which the number was noticeably above the diploid, in some cases even over 80. (The haploid number =20.) I also noticed at the very outset that the chromosomes were of different sizes. Such division-figures occur rather abundantly. In most cases, however, the chromosomes are either so near one another or form such clusters that their number cannot always be determined, not to mention the fact that the structure of all the chromosomes cannot be seen. An examination of the chromosome numbers shows that among them are multiples of the haploid number (20). Thus we find oocytes with chromosome numbers that are di-, tri- and tetra-ploid, but only seldom do the numbers correspond exactly to the diploid or polyploid numbers mentioned. And most remarkable is the fact that numbers approximating thirty and fifty also occur. Since

Fig. 5. Drawing of the chromosomes represented in Fig. 4. The chromosomes in two sections following each other. All 40 chromosomes are seen. (About × 3300.)

the same ovary as contains oocytes with any of these chromosome numbers invariably also contains oocytes that have the haploid number 20, it is clear that polyploid individuals are out of the question. The point could not be decided until I had detected the atypical manner of the first maturation division related above.

All such stages are interkinesis. The fact that the number of chromosomes varies so noticeably is due to the abnormal character of this interkinesis. Instead of a polar body being produced, into which the other halves of the bivalents would have moved, all the halves of the bivalents have remained in the egg cell. They may in very few cases form normal division-figures, as we noticed above, but in most cases this does not happen. Sometimes we see that part of the chromosomes form the metaphase plate of the second maturation division with its spindle-fibres, and sometimes in the same cell there can be 2 or even 3 deficiently developed spindles, but no distinct second meta-

phase develops. Instead, there may occur equational divisions of double chromosomes (two chromatids) into single chromosomes (one chromatid), and between these a so-called secondary pairing often occurs, which leads to the development of multivalent chromosome groups. It also often happens that the bivalents do not all divide into double chromosomes but remain as they are in the cell. The result of all this is that we see simultaneously in one cell bivalent, double and single chromosomes (Fig. 3).

What is the further development of such an abnormal egg cell? In the same way as an egg cell with a normal set of chromosomes, such an abnormal egg cell can also be freed from its follicle, come into the oviduct and be fertilized there. The chromosomes of the abnormal egg cell are generally partly single, partly double, and among them there may also be bivalents. In the egg cell there may be two or more deficiently developed metaphase spindles of the second maturation division, or there may be no spindle at all. With the spermatozoön the egg cell receives one more haploid set of chromosomes. Does another polar body develop now? It may be possible. But even if it is produced and thus some of the chromosomes of the egg cell should pass into the second polar body, the zygote would not be normal as regards its chromosome set, but would have additional chromosomes. The further development of the zygote depends upon how much and in what manner its chromosome set differs from the normal. Some very rare cases can lead to the development of triploid individuals. Mostly, however, this kind of an egg cell is an abortive egg cell, in which the chromosomal divergences are so great that the cell divisions are not possible for any length of time, and there does not exist any proper cytological basis for the differentiation of all the tissues. Therefore, the development of the egg cell mostly ceases already during the initial period of the pregnancy.

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CYTOLOGICAL STUDIES OF EXTRA FRAGMENT CHROMOSOMES IN RYE

III. THE MECHANISM OF NON-DISJUNCTION AT THE POLLEN MITOSIS

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THE material of the present investigation is the two strains of rye with extra fragment chromosomes described in previous publications (MÜNTZING, 1943, 1944, 1945). Each strain was derived from a single individual with $2n = 14 + 2$ f, one of these original plants being detected in the variety Östgöta Gråråg (MÜNTZING and PRAKKEN, 1941), the other one in the variety Vasa II. In both strains the fragment chromosomes are of the same general type and also of the same type as the fragments occurring in rye populations of other countries. They have a subterminal centromere and are of about half the size of the ordinary chromosomes. In Vasa II, however, the fragment was found to be slightly but significantly larger than in Östgöta Gråråg. It is not yet known whether this difference is structural or due to genotypical control. At any rate, the size difference is correlated to a very marked difference in the degree of pairing at first metaphase of meiosis. In plants of Vasa II with $2n = 14 + 2$ f the fragments were paired in 89 per cent of the p. m. c., the corresponding figure in Östgöta Gråråg being as low as 38 per cent.

The genetical effects of a varying number of such »standard» fragments were studied in Östgöta Gråråg. A slight but significant effect on the vegetative development could be demonstrated, but in all cases the effect was only obvious when several fragments were present. Fertility was found to be more influenced than the vegetative development. A marked and significant, though non-linear, negative correlation between number of fragments and fertility (male as well as female) was established. Considering the size and number of the fragments and the effects of whole extra chromosomes, it was concluded that the fragments are probably sub-inert, having a lower proportion of active genes than ordinary chromosomes.

Further possibilities of cytological analysis were obtained by the appearance of new types of fragment chromosomes in the Östgöta

Gråråg strain. Evidence was obtained that these new types are iso-fragments, having arisen by misdivision of the original standard fragment. Of the two new fragment types secured the large iso-fragment corresponds to twice the long arm of the standard fragment. In the same way the small iso-fragment probably corresponds to twice the short arm of the standard fragment.

A very peculiar feature of the standard fragment is its ability of numerical increase by a process of directed non-disjunction. On the male side this process occurs at the first pollen mitosis as first observed by HASEGAWA (1934). The experimental results demonstrate that this process is equally well developed in the ovules.

The transmission of the large and small iso-fragments was found to be quite different. As shown by the experimental data, the large iso-fragment has quite the same ability of multiplication as the standard fragment. The small iso-fragment, on the contrary, entirely lacks this mechanism of numerical increase.

It seemed desirable to study this differential behaviour by direct observations of the pollen mitosis. At the same time more insight might be gained into the process of non-disjunction of the standard fragment. Therefore anthers of plants representing the various fragment types were fixed in Carnoy in June, 1945. The anthers were preserved in alcohol (70 %) and then used for acetocarmine smears. It was also found to be possible and convenient to embed the anthers in paraffin and to make permanent slides after Feulgen staining [1]. All data reported below, however, are from fresh or semipermanent acetocarmine smears, which were made in about the same way as HASEGAWA's slides (HASEGAWA, 1934) and before the advantages of the permanent slides were realized. Thus, though an improved technique may facilitate a more detailed analysis later on, the present data are sufficient to elucidate the main features of the pollen mitosis in plants with standard fragments, large and small iso-fragments. The main problem is the mechanism of non-disjunction, but at the same time some data on the metaphase of the first pollen mitosis may also be given.

I. METAPHASE OBSERVATIONS.

In the acetocarmine smears only one anther was used for each slide. Even if this anther was at an optimal stage only a small fraction

[1] I am much indebted to my colleagues Dr. A. LEVAN and Dr. G. ÖSTERGREN for valuable technical suggestions.

of the numerous pollen grains contained good metaphase plates. As a rule, however, 10—20 analysable metaphases per slide could be found. Data are available for plants with 2 and 4 standard fragments and from plants with small and large iso-fragments.

1. PLANTS WITH 2 STANDARD FRAGMENTS.

Four different plants of Östgöta Gråråg were examined, the following result being obtained (Table 1):

TABLE 1. *Metaphase of the first pollen mitosis in plants with* $2n = 14 + 2$ *s. f.*

Plant and slide number	Number of fragments					Percentage of metaphases with fragments	Percentage of binucleate pollen grains
	0	1 s. f.	2 s. f.	1 s.f.+ 1 large iso-f.	1 small iso-f.		
2468, slide 1				1		—	—
» » 2	1	8	4	1		92	83
» » 3	6	7				53	23
» » 4	6	9				60	45
» » 5	13	8	—	—	1	40	23
» » 6	7	7				50	31
» » 7	5	6				54	25
» » 8	2	11	1			85	52
2468, total	40	56	5	2	1		
2472, slide 1	2	1				—	—
» » 2	4	12				75	23
» » 3	2	9	1			—	—
2472, total	8	22	1				
2490, slide 1	6	11				—	—
2460, » 1	8	10	2			60	56
Total of all plants	62	99	8	2	1		

The total values of the whole material represent 172 metaphases. The maximum class (99) had 7 ordinary chromosomes + 1 standard fragment (Fig. 10). This, of course, is according to expectation in a plant with $14 + 2$ s. f. However, owing to the bad pairing and the ensuing elimination of the fragments in this variety, the high frequency of pollen grains without fragments (62) is not unexpected. Non-conjunction of the fragments will also in some cases lead to the inclusion

of both fragments in the same pollen grain. Eight pollen grains of this kind were observed. In addition to the categories mentioned there were 3 pollen grains containing new fragment types. These were easily recognized as iso-fragments of quite the same kind as the small and large iso-fragments which had previously arisen in my cultures. Thus, the formation of iso-fragments from the standard fragment seems to be a regular though infrequent process. It was also observed twice in plants with $2n = 14 + 4$ s. f. to be discussed below.

In one of the plants with 2 standard fragments (2468) the chromosomal constitution at metaphase of the pollen mitosis was studied in 8 slides representing 8 different anthers. As in *Sorghum* (DARLINGTON and THOMAS, 1941) it was observed that the pollen grains carrying fragments were on an average slower in development than pollen grains without fragments. As a measure of the developmental stage of the anther the percentage of binucleate pollen grains of each anther was calculated. If this percentage is high, the metaphases still occurring in the anther are the latest ones and vice versa. Though data of this kind were only available for 7 different anthers, and the number of analysable metaphases in each anther was low, a significant positive correlation was found between the percentage of metaphase plates containing fragments and the percentage of binucleate pollen grains. The latter values are based on counts of about 200 pollen grains from each slide. The two series of percentage values of Table 1 give a coefficient of correlation as high as $+ 0{,}91$. A test of the significance of this coefficient according to the formula $t^2 = \frac{(n-2)r^2}{1-r^2}$ gives a t^2 of $24{,}09$ which corresponds to a P intermediate between $0{,}01$ and $0{,}001$.

In plant 2472 (Table 1) a total ratio of 8 mitoses without fragments: 23 mitoses with 1 or 2 fragments was obtained. This may seem to represent a higher proportion of mitoses with fragments than the total ratio 40 : 64 observed in the previous plant 2468. A χ^2 test, however, shows that the difference is not significant ($\chi^2 = 1{,}67$; $0{,}20 > P > 0{,}10$). A χ^2 test of the possible heterogeneity of the ratios of all four plants of Table 1 also gave a negative result.

In Table 1 the percentage values of metaphases with fragments and of binucleate pollen grains are also available for plant 2472, slide 2, and for plant 2460, slide 1. If these values are combined with the 7 pairs of percentage values obtained in plant 2468 the coefficient of correlation will be $+ 0{,}72$, $t^2 = 8{,}61$ and P intermediate between $0{,}05$ and $0{,}02$. The lower significance in this case as compared to the values

obtained for plant 2468 alone ($r = +0{,}91$, $t^2 = 24{,}09$ and P intermediate between 0,01 and 0,001) may be due to environmental or genetical differences. Plant 2468 was grown in a rather dark and dry greenhouse, the other plants examined in the open air.

The plants with $2n = 14 + 2$ s. f. so far discussed all belonged to the variety Östgöta Gråråg. In the other variety, Vasa II, the metaphase of the pollen mitosis has only been observed in a single slide. In plant 2494, having $2n = 14 + 2$ fragments, 9 pollen metaphases all contained 7 chromosomes + 1 fragment. This is in accordance with the good meiotic fragment pairing in Vasa II. The appearance of the fragment (Fig. 12) was about the same as in Östgöta Gråråg.

2. PLANTS WITH 4 STANDARD FRAGMENTS.

Chromosome counts at the metaphase of the pollen mitosis were undertaken in 4 different plants of Östgöta Gråråg having $2n = 14 + 4$ s. f. The following results were obtained:

Plant number	*Chromosome number*						
	7	7+1 s. f.	7+2 s. f.	7+3 s. f.	7+2 s. f. +1 iso-f.	7+4 s. f.	7+3 s. f. +1 small iso-f.
2484, slide 1		1	5	—	—	—	1
2459, » 1			2	—	1		
2487, » 1			5	3	—	1	
2488, » 1	1	9	4				
» , » 2		9	5				
Total	1	19	21	3	1	1	1

As expected in a material with bad meiotic pairing of the fragments, the average fragment number at the pollen mitosis ($M = 1{,}72$) is lower than half the somatic number. This is certainly due to meiotic elimination. In 2 mitoses of 47 new fragment types appeared, in one case a small iso-fragment of the usual type, in the other (Fig. 11) possibly a new type of iso-fragment. Owing to the very marked chromosome contraction in this cell it is not excluded, however, that it is a question of the usual large iso-fragment. In the same cell there are 2 standard fragments besides the 7 ordinary chromosomes.

The apparent heterogeneity of the chromosome numbers of the different plants and slides may in part be due to differences in the stage of development of the anthers, just as in the plants with $14 + 2$ s. f. The numbers available, however, are too small for statistical calculations of this kind.

3. PLANTS WITH ISO-FRAGMENTS.

The first pollen mitosis was observed in 4 plants with 2n = 14 + + 1 small iso-fragment. A total of 51 metaphases were examined and of these 46 had 7 chromosomes and no fragments, the remaining 5 containing one typical small iso-fragment in addition to the seven ordinary chromosomes (Fig. 13). The separate ratios in the 4 plants were 12 : 0; 7 : 1; 18 : 2 and 9 : 2. The low proportion of pollen metaphases with fragments is certainly due to a rather high degree of meiotic elimination of the small and unpaired fragment.

In plants with 2n = 14 + 2 large iso-fragments the frequency of fragments at the pollen mitosis was rather high. The following values were obtained:

Plant number	*Chromosome number*			*M*	*Percentage of binucleate grains*
	7	8	9		
2456, slide 1		12	7	8,37	50
2492, » 1		7	6	8,46	89
» , » 2	4	9	1	7,79	43
Total	4	28	14	8,22	

In the pollen mitosis, just as in somatic plates, the large iso-fragments could not always be distinguished from the other chromosomes. However, the chromosome numbers 8 and 9 in the above table certainly correspond to 7 + 1 large iso-f. and 7 + 2 large iso-f. respectively. The average fragment number, 1,22, is rather high. This may in part be due to the fact that all three slides examined represent a rather advanced stage of development, the percentages of binucleate grains ranging from 43 to 89. The figures again indicate that the rate of development of the pollen grains is retarded by the presence of fragments.

II. THE MECHANISM OF NON-DISJUNCTION AT ANAPHASE.

1. THE STANDARD FRAGMENTS.

Good anaphases of the first pollen mitosis are as rare as good metaphases, but a sufficient number of cells at this stage were seen to elucidate the main behaviour of the various fragment types. In several cases it proved to be very useful to turn the pollen grain around by pushing the cover slip.

The behaviour of the standard fragments in two plants (2468 and

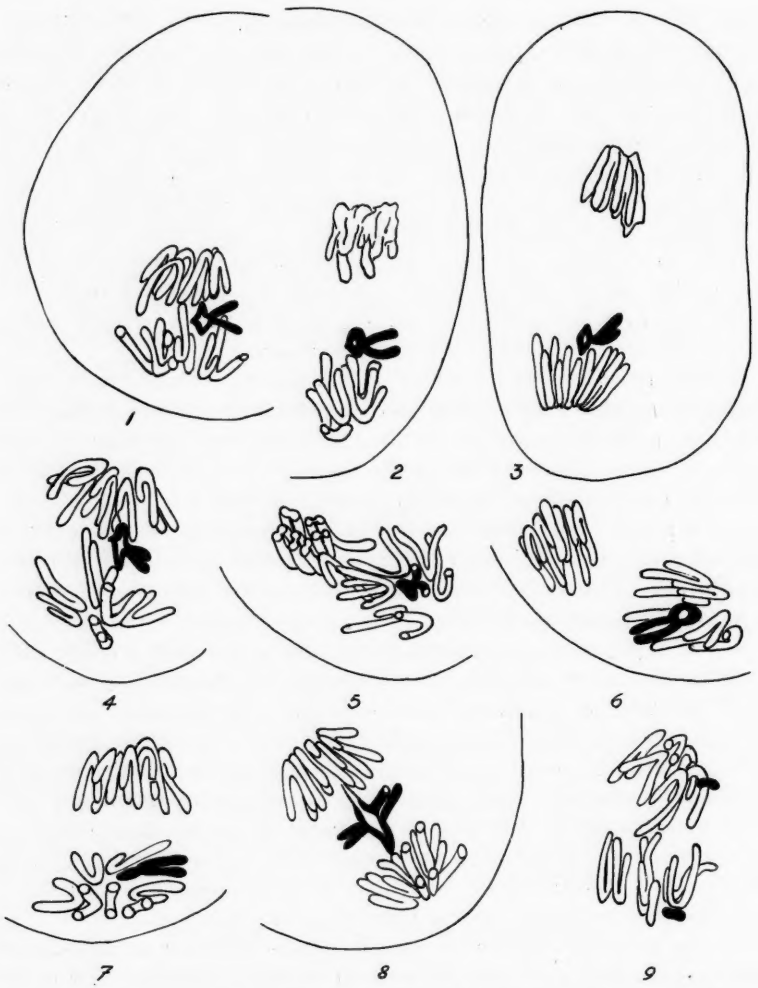
2472) with $2n = 14 + 2$ s. f. is illustrated by Figs. 1—5. The anaphase represented by Fig. 1 shows a very characteristic structure situated exactly between the anaphase groups. This structure looks like a bivalent with two localized chiasmata near the centromere, these chiasmata being situated at the same distance on either side of the centromere. Undoubtedly, however, the peculiar formation corresponds to the standard fragment. The centromere has divided and is trying to pull the two chromatids apart, but resistance to this separation is offered in a special segment of the long arm not far from the centromere. The chromatids also stick together at the end of the short arm. The distal portions of the long arm, on the other hand, have succeeded in separating and are quite free from each other.

This behaviour and position of the standard fragment at early anaphase is quite characteristic and was observed in many cells. Ten separate standard fragments at this stage are pictured in Figs. 14—23. They represent somewhat different degrees of earliness, judging from the different degrees of stretching in the centromere region. In some cases the force separating the two centromeres was so strong as to attenuate the chromosome strands near the centromere to thin threads. In Figs. 17—19 the ring-shaped part involving the centromere region is seen more or less in side view.

Fig. 2 represents a somewhat later anaphase than Fig. 1. The spindle is longer and the characteristic configuration of the two chromatids of the standard fragment is now situated near the anaphase group passing to the generative pole. In Fig. 3 the process has proceeded still further. This is late anaphase with a long spindle and the standard fragment configuration situated close to the generative anaphase group. Fig. 4 (from plant 2468) represents about the same stage as Fig. 1 (from plant 2472). In the fragment configuration the centromere region is stretched rather much. In each of the anaphase groups the 7 ordinary chromosomes may be distinguished.

Fig. 5 shows a rather frequent deviation from the normal course, the spindle being bent and not parallel to the longitudinal axis of the grain. In one of the anaphase groups, the one probably giving rise to the vegetative nucleus, there are 7 ordinary chromosomes, 6 of which are seen in polar view. In the other group, probably representing the generative pole, the typical standard fragment configuration is lying among the 7 ordinary chromosomes.

In plants of the variety Vasa II, having $2n = 14 + 2$ fragments, quite a similar behaviour of the fragment was observed to that found



Figs. 1—5, anaphase of the first pollen division in plants of Östgöta Gråråg with $2n = 14 + 2$ s. f. — Fig. 1, early anaphase, typical fragment configuration lagging between the anaphase groups. — Fig. 2, a somewhat later stage than in Fig. 1, the fragment configuration near the generative anaphase group. — Fig. 3, late anaphase with a stretched spindle, the fragment configuration close to the generative group. — Fig. 4, typical fragment configuration showing a pronounced stretching in the centromere region, 7 chromosomes in each anaphase group. — Fig. 5, anaphase with a bent spindle, in one of the groups (probably the generative) 7 chromosomes + the fragment configuration, in the other group 7 chromosomes. — Figs. 6—7, anaphases

in the corresponding plants of Östgöta Gråråg. Figs. 24—26 show the fragment configuration at anaphase in three different cells of a Vasa II plant. The general appearance is the same as in Östgöta Gråråg (Figs. 14—23), but the free arms may be somewhat longer. Figs. 6—7 show complete anaphases in Vasa II. In Fig. 6 the spindle has a transverse position and is somewhat bent. In the group probably representing the generative pole there are 7 chromosomes + the typical fragment configuration. In the other group there is no fragment and probably 7 ordinary chromosomes. In Fig. 7 the anaphase is apparently rather early. The spindle is somewhat bent, however, and thus Fig. 7 may represent about the same stage as Fig. 6. In this case the spindle has a longitudinal position leaving no doubt about the position of the vegetative and generative pole. In the vegetative group the 7 chromosomes could be distinguished and among them the satellite chromosome. In the generative group there are again 7 chromosomes plus the typical fragment configuration. The ring-shaped region of this configuration is seen in side view.

Judging from the data so far available, the fragment of Vasa II seems to reach the generative pole earlier than in Östgöta Gråråg and does not lag so much at the equator as in the latter variety. Be that as it may, also in Östgöta Gråråg the fragment configuration is certainly in most cases included in the generative nucleus. This view is supported by observations of the degree of lagging and chromosome elimination at anaphase and telophase.

In three plants of Östgöta Gråråg having $2n = 14 + 2$ s. f. a total of 113 anaphases were observed in 7 slides. Of these, 51 were apparently regular, while 62 contained lagging fragments. At metaphase a total of 172 cells comprised 62 without fragments and 110 with fragments (Table 1). The distributions 51 : 62 and 62 : 110 are not significantly different. Nevertheless, the higher proportion of metaphases with fragments than anaphases with lagging fragments indicates that some fragments pass to the pole without lagging and are present in the apparently regular anaphases. One such case was, indeed, observed in Östgöta Gråråg (Fig. 5) and several cases in Vasa II (Figs. 6—7). In the latter variety the anaphase observations are limited to 14 cells. Four of these

in plants of Vasa II with $2n = 14 + 2$ f., in both cells 7 chromosomes + the fragment configuration in one of the groups, 7 chromosomes in the other. — Fig. 8, anaphase in a plant with $2n = 14 + 2$ large iso-fragments, 7 chromosomes in each anaphase group and a lagging fragment configuration with attenuated centromere region and 4 free arms in the middle. — Fig. 9, anaphase in a plant with $2n = 14 + 1$ small iso-f., normal division of the fragment. — × 1600.

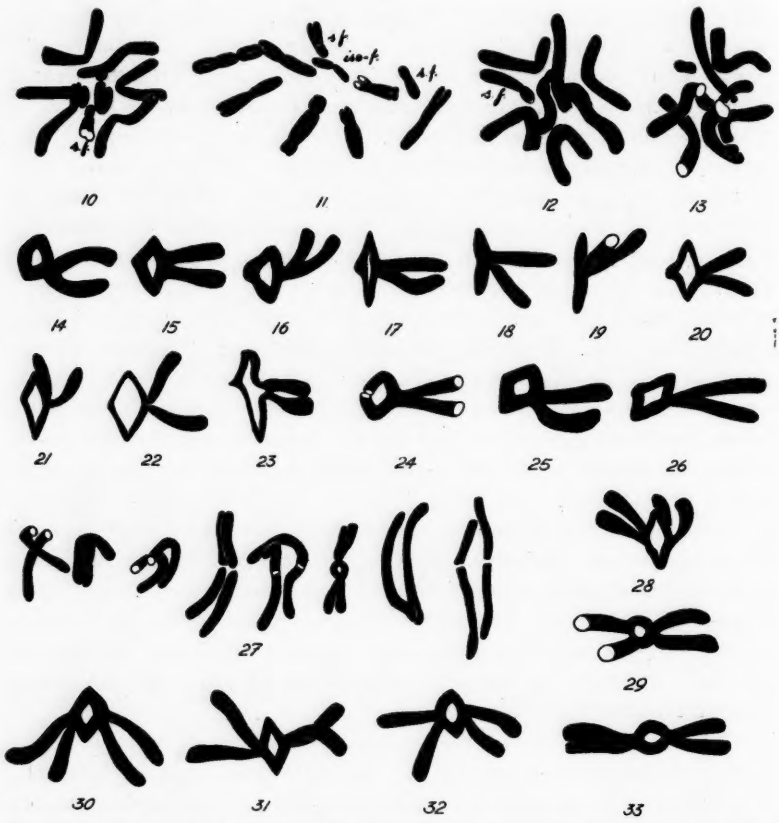


Fig. 10, pollen metaphase of an Östgöta Gråråg plant showing 7 chromosomes + 1 s. f. — Fig. 11, pollen metaphase in a plant of the same variety having 2n = 14 + 4 s. f., 7 ordinary chromosomes, 2 standard fragments and 1 iso-fragment in the plate. — Fig. 12, pollen metaphase in Vasa II showing 7 chromosomes + 1 s. f. — Fig. 13, pollen metaphase in a plant with a small iso-fragment. — Figs. 14—23, fragment configurations at anaphase (separately drawn) in plants of Östgöta Gråråg having 2n = = 14 + 2 s. f. — Figs. 24—26, the same in a plant of Vasa II. — Fig. 27, a commencing pollen anaphase in a plant with 2n = 14 + 2 large iso-f., the 7 ordinary chromosomes + 1 large iso-f. separately drawn. — Figs. 28—33, large iso-fragments (separately drawn) lagging at anaphase of the pollen division. — Figs. 10, 12, 13, 27, × 1500; Fig. 11, × 1300; Figs. 14—26 and 28—33, × 2800.

contained lagging fragments, in 4 other cells the fragment was visible in the generative anaphase group. In 6 cells, finally, the anaphases were apparently regular but the fragment had probably been contained

in one of the anaphase groups. This probability is supported by the fact that the fragment was present in all pollen metaphases observed in this variety.

The fragments lagging at anaphase are evidently in most cases included in the generative nucleus. This is indicated by Figs. 1—3 and further supported by telophase observations. In the seven slides used for the anaphase studies a total of 209 telophases were also observed. Only 10 of these telophases contained eliminated or lagging fragments, the remaining 199 telophases being quite regular. The same regularity at telophase was also observed in a slide of a Vasa II plant with $2n = 14 + 2$ f.

In plants of Östgöta Gråråg with 4 standard fragments the ana- and telo-phases were somewhat more irregular. Of 17 anaphases observed 7 were regular, 7 contained lagging fragments and 3 were irregular in other respects. Of 54 telophases examined 37 were quite regular, 15 contained bridges or protruding chromosome arms and in 2 cells there were eliminated or lagging chromosomes. Thus, also in this category of plants telophase is more regular than anaphase, showing that the fragments lagging at anaphase are generally included in one of the telophase nuclei.

2. THE ISO-FRAGMENTS.

The behaviour of the large iso-fragment at anaphase of the pollen mitosis is of quite the same type as that of the standard fragment. The only difference is the presence of four free arms instead of two in the structure lagging at anaphase. Fig. 8 shows a typical case. In both anaphase groups there are 7 ordinary chromosomes and at the equator the large iso-fragment tries to divide. The centromere division is completed and the force causing the centromeres to move towards the poles has attenuated the chromosome segments near the centromeres to rather thin threads. Separation of the chromatids is prevented at the same place as in the long arm of the standard fragment. In the large iso-fragments there are two such places, the iso-fragment being composed of twice the long arm of the standard fragment. These arms are in a mirror image position and this mirror image is also quite evident in the structure lagging at anaphase.

Figs. 28—33 show 6 separate structures of this kind. The large iso-fragments in question all show four free arms, and in all cases the centromere has divided. The ring-shaped part around the centromere region shows different degrees of attenuation, probably corresponding

to different degrees of earliness. The structure represented in Fig. 33 is also present in Fig. 27 together with the rest of the chromosome complement of this cell. This cell represents a just commencing anaphase, in which the chromatids have separated in all of the ordinary chromosomes. In 6 of these 7 chromosomes the centromere has also divided. In the large iso-fragment the centromere has likewise divided and most probably at the same time as the centromeres of the other chromosomes. The chromatids of the iso-fragment have also separated, except in the rather sharply defined segments on both sides of the centromere.

A comparison between anaphase and telophase in the pollen of plants with $2n = 14 + 2$ large iso-fragments shows that the fragments behave in a similar way as the standard fragments. Of 30 anaphases observed (in 3 slides of 2 plants) 17 were apparently regular, 13 showing lagging iso-fragments. Of 50 telophases 40 were regular, 5 contained bridges or protruding chromosome arms and 5 contained eliminated or lagging chromosomes. As the frequency of iso-fragments present at metaphase was rather high (p. 102), several of the apparently normal anaphases must have contained fragments. This is the same situation as in the plants carrying 2 standard fragments.

Owing to meiotic elimination the small iso-fragment in plants with one such fragment is rare at metaphase of the pollen mitosis (p. 102). It is also rare at anaphase and was only observed at this stage in 7 cells of 151. These total values were gathered from 4 slides, representing 4 different plants. In contrast to the standard fragment and the large iso-fragment the small iso-fragment divides quite regularly, as may be seen from Fig. 9. In this cell there is a normal 7—7 distribution of the ordinary chromosomes and one small iso-fragment in each anaphase group. Telophase in the same plants was absolutely regular, a total of 74 cells being observed.

III. COMPARISON BETWEEN EXPERIMENTAL DATA AND CYTOLOGICAL OBSERVATIONS.

It is of interest to compare the frequency and behaviour of the different fragment types at the pollen mitosis with their transmission in various cross combinations (MÜNTZING, 1945).

According to Table 1, a total of 172 pollen metaphases in plants with $2n = 14 + 2$ s. f. comprised 62 metaphases with 0 fragments, 100 with 1 and 10 with 2 fragments. The percentage values of the three categories will be 36,0, 58,1 and 5,8 respectively. Owing to non-dis-

junction metaphases with 1 fragment should give male gametes with 2 fragments and metaphases with 2 fragments should result in gametes with 4 fragments. From the cross combination $14 \times 14 + 2$ s. f. the chromosome numbers of 147 plants have been determined. These plants are the 46 plants mentioned on p. 460 in the paper cited plus new progenies recently studied. The chromosomal variation in this material was the following:

Chromosome numbers:	14	14 + 1 f.	14 + 2 f.	14 + 3 f.	14 + 4 f.
Observed:	62	19	62	—	4
Expected:	52,92	—	85,41	—	8,53

The expected values are based on the percentage values just given. The main difference between observation and expectation is the occurrence of 19 plants with one fragment instead of two. This shows that the process of non-disjunction, though quite predominant, is not absolute. It is possible that in some cases the standard fragment divides at the same time as the other chromosomes or only slightly later. Such a division, however, was not observed. Another possibility are disturbances of some kind at the second pollen mitosis. So far it has not been possible to study this division in rye.

Besides the occurrence of plants with 14 + 1 f. another discrepancy between observation and expectation is a slight excess of plants without fragments and too few plants with fragments. This difference is too slight to be statistically significant, but other data point in the same direction and indicate that gametes or zygotes carrying several extra chromosomes are less viable than those without fragments or with a low number of fragments.

Thus, in the offspring of plants with $2n = 14 + 2$ s. f., raised from isolation or intercrossing, the frequency of fragments is lower than would be expected from observation of the pollen mitosis in such plants. In the previous paper (1945, pp. 461—462) the chromosome numbers are given for progenies of intercrossed or selfed plants with 2 standard fragments. If the two series are added, the following total series will be obtained:

Number of fragments:	0	1	2	3	4	5	6	7	8
Observed:	37	9	78	15	32	2	—	—	—
Expected:	22,42	—	72,38	—	65,64	—	11,66	—	0,59

The expected values are again based on the observations of the pollen metaphase and the percentage values 36,0 (0 fragments), 58,1

(2 fragments) and 5,8 (4 fragments). Assuming the same ratio to occur also on the female side, the number of fragments in the offspring would range from 0 to 8 in the frequencies given. The assumption of a similar ratio in the ovules as in the pollen is in the main supported by the results obtained in reciprocal crosses between plants with no fragments and plants with 2 standard fragments (MÜNTZING, 1945, pp. 459—460 and unpublished data).

A comparison between the observed and expected values shows a marked deficit of plants with 4 or more fragments and a surplus of plants with 0 to 3 fragments. A χ^2 test combining the data into the fragment number classes 0, 1—2, 3—4 and 5—8 gave a χ^2 of 26,61 and a P smaller than 0,001.

Similar calculations may also be made on basis of the observations made in plants with $2n = 14 + 4$ s. f. As reported above, the number of fragments at metaphase of the pollen mitosis ranged from 0 to 4. If these fragments undergo non-disjunction and pass to the generative pole the gametes will have 0, 2, 4, 6 and 8 fragments in the following frequencies (based on the data given on p. 101): 2,1 (0 f.), 40,4 (2 f.), 44,7 (4 f.), 8,5 (6 f.) and 4,3 (8 f.). Assuming the same frequencies in the ovules, the percentages of individuals with different numbers of fragments in the offspring of selfed or intercrossed plants with 14 + 4 s. f. may be calculated. Theoretically, the fragment number should range from 0 to 16. Two progenies of this kind have been studied previously (MÜNTZING, 1945, p. 463), the total number of plants amounting to 28. The values obtained and expected are as follows:

Number of fragments:	0	1	2	3	4	5	6	7—16	n
Observed:	3	1	6	1	9	0	8	0	28
Expected:			5,58				10,21	12,20	27,99

In order to prevent expected values lower than 5 the classes 0 to 4, 5 to 6 and 7 to 16 had to be added when making a χ^2 test. In this way χ^2 will be 49,95 and $P < 0{,}001$, thus again demonstrating a marked surplus of plants with low number of fragments and a deficit of plants with high numbers. This deficit may to some extent be caused by the irregularities observed to occur at the pollen mitosis, but the most important cause is probably a reduced viability of gametes carrying many fragments.

It remains to point out that the behaviour of the iso-fragment at the pollen mitosis is entirely in accordance with the experimental data.

Thus, in crosses of the type 14 × 14 + 1 large iso-fragment (MÜNTZING, 1945, p. 465) the large iso-fragment, when present, was always duplicated. The non-disjunction at the pollen mitosis necessary to explain this result has now actually been observed. Inversely, the experimental results (l. c., pp. 464—465) showed that the small iso-fragment lacked the ability of duplication by means of non-disjunction. It was also observed to divide at the pollen mitosis as regularly as the ordinary chromosomes. The experimental data called for a frequency of about 7,5 per cent of pollen grains with small iso-fragments. Of 51 pollen metaphases observed 5 (i. e. 10 per cent) contained the fragment. Thus, expectation and observation are in good agreement.

IV. THE OCCURRENCE OF CHROMATIN DROPS.

In plants with fragments a rather frequent occurrence of one or two spherical bodies was observed at all stages of the first pollen mitosis. As a rule these bodies were lying in the plasma, but sometimes they were observed to be in close contact with the nuclear membrane in uninucleate pollen grains or even to lie inside the nucleus. These bodies had quite the same colour as the chromosomes in acetocarmine smears, and in a permanent smear stained with Feulgen they were stained in the same way as the ordinary chromosomes. The simplest explanation would be that these chromatin drops are eliminated fragment chromosomes. This explanation is supported by the fact that such bodies were either entirely absent or rather rare in plants without fragments. A certain proportionality was also established between fragment size and drop size in plants with small iso-fragments, standard fragments and large iso-fragments. On the other hand, plants with $2n = 14 + 4$ s. f. were found to have a significantly lower frequency of drops than plants with $2n = 14 + 2$ s. f. This is incompatible with the elimination theory, as plants with 4 standard fragments show more meiotic irregularities than plants with 2 standard fragments. Further, in both these categories of plants the frequency of drops was significantly higher in binucleate than in uninucleate pollen grains, indicating that chromatin drops are set free during the pollen mitosis. The whole story is at present unsolved, and therefore publication of the details must be deferred until more data have been gathered. However, the phenomenon is very characteristic and is therefore mentioned already now.

V. DISCUSSION.

The pollen mitosis in rye plants with extra fragment chromosomes has previously been studied by HASEGAWA (1934). In a plant with $2n = 16$ he examined a total of 61 metaphases of the first pollen division and found 60 of them to contain 7 ordinary chromosomes plus one fragment of just the same type as the standard fragment in my material. The material observed also included one metaphase with 2 such extra fragments, but not a single metaphase without fragment was seen. This is evidently the same situation as is met with in corresponding plants of the variety Vasa II. Just as in HASEGAWA's material, a regular meiotic pairing leads to a regular occurrence of one fragment at the first pollen mitosis. In Östgöta Gråråg, on the contrary, the fragment number at this stage was found to be more variable owing to the poor meiotic pairing of the fragments in this variety.

At anaphase of the first pollen division HASEGAWA found that extra fragment chromosomes show a rather irregular behaviour and that they often reach the poles later than the other chromosomes. In some cases, he says, the split halves of the extra chromosomes are carried to either pole but in most cases they are included in the generative nucleus. This statement is supported by his Table 2 (l. c., p. 74), giving data from 21 different anaphases and 138 telophases. It is also supported by his figures 8 and 14, showing two parallel but separate chromatids near the generative pole.

Thus, the typical configuration composed of the two non-disjoining chromatids of the standard fragment was not seen or realized by HASEGAWA. The mechanism described in the present paper occurred in both the varieties tested, and the fragments present in these varieties are morphologically of just the same kind as those occurring in HASEGAWA's material and in other rye populations. Therefore it is highly probable that the same mechanism also occurred in the Japanese material, though the cells observed were not clear enough to reveal the true situation. The laggards in HASEGAWA's figures 8, 12 and 14 may in reality have been of the same kind as those described in the present paper. Only his Fig. 13 indicates a true division of the fragment chromosome.

In the great majority of cases the two chromatids of the standard fragment, passing to the generative nucleus, still stick together at late anaphase. Though their later fate could not be directly observed, it is safe to assume, with support from the experimental data, that the two

chromatids finally separate in the resting nucleus and then divide normally in the second pollen division.

The behaviour of the standard fragments at the first pollen mitosis and at a corresponding stage in the ovules is quite remarkable. The process of non-disjunction just occurs one single time during each life cycle, the division of the fragment otherwise proceeding quite regularly. This must be concluded from the fact that the same fragment number is found in the root tips of the seedlings as at meiosis in the adult plants. As the process of non-disjunction is only shown by the fragment and not by the ordinary chromosomes, the fragment must have some special property leading to this differential behaviour. The cause might be a stickiness of the fragment or a lack of synchronization between the centromere divisions and the lapse of chromatid attraction. Another possibility is a failure of reproduction in one or more special segments. All these possibilities require not only a special chemical constitution of the fragment itself but also a special reaction with the environment present in the pollen grains and the ovules.

At the pollen mitosis in *Uvularia* BARBER (1941) observed a strong tendency of the centromeres to divide before the chromatids are ready for separation. The centromeres are divided already at metaphase, and the distance between the centromeres gradually increases even before the main bulk of the chromatids separate. This causes the formation of configurations strikingly like those in bivalents with two chiasmata close on either side of the centromere. These configurations are also rather similar to the rye fragments lagging at the anaphase of the first pollen division, but there is an important difference. In rye, in contrast to *Uvularia*, the chromatids only stick together at short segments on both sides of the centromere, invariably leaving two long free arms. This strongly indicates the presence of special segments near the centromere, in which reproduction is delayed. As nucleic acid attachment is necessary for reproduction (cf. DARLINGTON, 1942), these special segments may be supposed to be heterochromatic and subject to nucleic acid starvation at the pollen mitosis.

The view that chromatid separation in the standard fragment is controlled by special segments near the centromere is supported by the behaviour of the large iso-fragments. In these fragments the ring-shaped part including the centromere was of just the same type and size as in the standard fragment. The only difference is that in the large iso-fragment four free arms instead of two are attached to the ring. If the difficulty of chromatid separation had been due to a lack of syn-

chronization or to a general stickiness, the central ring should have been quite variable in size and sometimes much larger than now observed. Thus, the present observations strongly indicate a differentiation in segments with different ability or rate of reproduction and separation.

A puzzling question is the behaviour of the small iso-fragment which according to experimental results as well as direct observation divides quite normally at the pollen mitosis. There are very good reasons for the assumption that this fragment is made up of the short arm of the standard fragment. This arm shows difficulty of separation in the standard fragment but not in the corresponding iso-fragment. This different behaviour is at present inexplicable.

The present observations of the pollen mitosis and the stable transmission of the fragments in vegetative divisions strongly indicate that the centromere of the standard fragment as well as those of the large and small iso-fragments are just as efficient as the centromeres of the ordinary chromosomes. Non-disjunction at the pollen mitosis is not caused by defective centromeres with a delayed division. On the contrary, the centromeres of the fragments divide at the same time as those of the other chromosomes, and when resistance is raised to their passage to the poles, they are strong enough to attenuate the chromosome segments between the centromere and the point of non-separation to thin threads. This was observed not only in the standard fragment but also in the newly arisen large iso-fragment.

According to the highly interesting theories of DARLINGTON (1939, 1940) concerning the structure of the centromere and the origin of iso-chromosomes by misdivision, the centromere is a compound structure containing several or many centrogenes. DARLINGTON suggests that large chromosomes may require a larger aggregation of centrogenes than small ones. He also points out that if centrogenes are multiple, the possibility also arises that misdivision may divide them in every proportion from all-to-none to one-to-one. Consequently the resulting fragments should receive a variable number of centrogenes and, thus, the strength of their centromeres should be variable.

In the rye material iso-fragments repeatedly arise from the standard fragment, and hence it might be possible to obtain iso-fragments showing differences as to the effectiveness of their centromeres. The fragments having too weak centromeres, however, will probably disappear before they can be observed. The iso-fragments now present in our strains may represent a plus selection, i.e. a doubling of at least half the centrogenes of the standard fragment.

In *Nicandra* DARLINGTON and JANAKI-AMMAL (1945) found that the centromeres of a pair of iso-chromosomes must be weaker than in the other chromosomes. Both as bivalents and as quadrivalents (in tetraploid derivatives) the iso-chromosomes lagged in first metaphase congression and in first anaphase separation. Even at mitosis in root-tips, anthers and ovaries a similar lagging was observed. Such observations evidently support the centrogene theory.

When discussing the origin of the iso-chromosomes and the strength of their centromeres the observations of SCHRADER (1936, 1939) on the centromere (the kinetochore) in Amphibia must be taken into consideration. The main point is that the centromere shows a double structure already at diakinesis and still more clearly at the metaphase of meiosis. Each chromatid has its own »spindle spherule», but these spherules do not separate from each other until anaphase. At this stage each of the two spherules was frequently seen to be connected with its own spindle element. Similar observations of a double structure of the centromere at mitosis have been made by ÖSTERGREN on plant material (unpublished). He has also evidence of a double centromere at the metaphase of meiosis (ÖSTERGREN, 1945 a). The observations of BARBER of an early centromere division in *Uvularia* were mentioned above. This early doubleness of the centromere may perhaps explain the origin of new chromosome types by misdivision without the assumption of a break *through* the centromere, i. e. in such a way that the supposed centrogenes are distributed in various proportions among the products of misdivision. It may instead be a question of abnormal attachment of the chromatid segments to the spindle spherules. Before the separate spindle spherules appear the meiotic chromsome at diplotene is composed of two chromatids attached to an undivided centromere region. At later stages when the spindle spherules become distinct one chromatid with its left and right arm will normally be attached to one of the two spherules and the other chromatid to the other spherule. In univalents or under abnormal circumstances it may possibly occur that the left arms of both chromatids are attached to one of the spherules and the two right arms to the other. This would give rise to telocentric chromosomes and later on to iso-chromosomes with quite normal centromeres of just the same strength as in the original chromosomes. Though not conclusive the results so far obtained in rye favour such an explanation.

The origin and function of the standard fragments occasionally

occurring in rye populations of widely different countries is still an unsolved problem. ÖSTERGREN (1945 b) has advanced good arguments for the view that these fragments are only deleterious and have a kind of parasitic existence. This view is in part based on previous experimental data (MÜNTZING, 1943) and is further supported to some extent by the present observations on the first pollen mitosis. Evidence was obtained that pollen grains with fragments are slower in development than those without fragments. This evidence may be combined with the previous observations that plants with fragments have a reduced pollen fertility as well as a reduced percentage of seed setting (l. c., pp. 102—105). The comparison of the chromosome counts at the pollen mitosis with the experimental data (p. 108) also indicated that gametes with fragments, and especially those with many fragments, are less functionable than gametes without fragments.

Recent data by RANDOLPH (1941), as cited by PATTERSON (1942)[1], on the B chromosomes in maize point in the same direction as in rye. According to RANDOLPH, the presence of the B chromosome is apparently neither necessary nor beneficial to maize, although several of these elements may be present without impairing viability or fertility. Very considerable reduplication of the B chromosome, however, has a detrimental effect on the system, lowering the viability and fertility of the plant as well as causing phenotypic effects. The B chromosome is said to be somewhat comparable to a virus, being parasitic, or even pathogenic, in its effect.

DARLINGTON and UPCOTT (1941) have shown that the B chromosomes in maize are liable to get eliminated at meiosis as well as mitosis. They are also specially liable to deletion and then give smaller, so-called b chromosomes. This double selection pressure, which tends to reduce their size and frequency in the population, must be compensated by an equal and opposite selection in their favour. This opposite selection, the details of which are not known, is supposed to depend on the heterochromatic nature of the B chromosomes and their importance in the nucleic acid metabolism of the cell.

So far, there is no reason to assume, except by analogy to the situation in maize, that the rye fragments have special functions of value for the plants carrying them. The special mechanism of post-meiotic non-disjunction described above is sufficient to explain that the fragments are retained in the populations in spite of their deleterious effects.

[1] The original paper is not yet available in Sweden.

SUMMARY.

(1) The first pollen mitosis was studied in rye plants with extra fragment chromosomes of different types (standard fragments, large and small iso-fragments). The frequency of fragments observed at metaphase was in conformity with the degree of meiotic elimination but higher than the frequency of fragments observed in the offspring. Gametes with fragments, especially those with several fragments, are evidently less viable than gametes without fragments.

(2) In a plant with 2 standard fragments a significant positive correlation was found between the percentage of metaphases with fragments and the percentage of binucleate pollen grains in the same slide. This shows that pollen grains carrying fragments are slower in development than those without fragments.

(3) At early anaphase the standard fragment in most cases lags between the anaphase groups and has the appearance of a bivalent with two free arms and two localized chiasmata near the centromere. This structure arises because the centromere has divided and tries to pull the two chromatids apart. Resistance to this separation is offered in a special, probably heterochromatic segment of the long arm not far from the centromere. The chromatids also stick together at the end of the short arm. As anaphase proceeds and the spindle stretches, the non-separating chromatids of the standard fragment in typical cases pass to the generative pole.

(4) The large iso-fragment behaves in the same way as the standard fragment, the only difference being the presence of four free arms instead of two in the structure lagging at anaphase. Separation of the chromatids is prevented at the same place as in the long arm of the standard fragment. In the large iso-fragment there are two such places, the iso-fragment being composed of twice the long arm of the standard fragment. These arms are in a mirror image position and this mirror image is also quite evident in the structure lagging at anaphase. The small iso-fragment, on the contrary, divides quite regularly and shows no evidence of non-disjunction.

(5) The centromere of the standard fragment as well as those of the large and small iso-fragment are just as efficient as the centromeres of the ordinary chromosomes. Non-disjunction at the pollen mitosis is not caused by defective centromeres with a delayed division. On the contrary, the centromeres of the fragments divide at the same time as those of the other chromosomes, and when resistance is raised to their

passage to the poles, they are strong enough to attenuate the chromosome segments between the centromere and the point of non-separation to thin threads. This was observed not only in the standard fragment but also in the newly arisen large iso-fragment. In the light of these observations the mechanism of misdivision is discussed. It is suggested that iso-chromosomes may arise from an abnormal attachment of chromatid segments to the halves of a normally divided centromere.

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DRASTIC MORPHOLOGICAL MUTATIONS IN BARLEY

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FOR several years the present author has gathered material on spontaneous and induced mutating in agricultural plants. Special model experiments have been performed with three strains of two-rowed barley (Golden, Maja and Ymer barley). In this paper only one set of results will be discussed, those concerning profound alterations in morphology and their effect on viability.

Vital mutations may be grouped either as morphological or physiological (GUSTAFSSON, 1941). The former imply qualitative changes and are easily classified in segregating progenies; the latter imply quantitative changes, relating to straw-height, ear-length, earliness, straw-stiffness, and so on. These two types cannot be sharply delimited, since transitions occur and many mutations are highly pleiotropic in character.

Some mutations of the morphological group are characterized by a peculiar bright-green colour of stem and leaves; they lack waxiness, are more or less »non-glaucous». In addition to this distinct change, some minor physiological properties may be altered. Generally speaking, however, pleiotropy is rather slight.

Another sub-group comprises the well-defined erectoides mutations, distinguished by their dense compact ears and stiff straws. Sometimes they deviate considerably from the parent lines, and pleiotropy is then obvious. Some attain great interest from a practical point of view.

Finally we have the group of »drastic» mutations. changing the phenotype profoundly, in many instances with respect to spikelet or flower structure. They break the morphological frame of the species or variety. Here the monsters are found, to use GOLDSCHMIDT's expression (1940). Some are sterile in homozygous condition, others are fertile and may be called vital, although in the cases examined the yield gets decreased. Changes of this types are always pleiotropic. They will be fully discussed in a later paper (GUSTAFSSON, 1946). Included in this sub-group are mut. bractoides, calcaroides, scirpoides, densinodo-

sum, intermedium, »lemma-like glumes» (GUSTAFSSON and ÅBERG, 1940), two-flowered mutations (l. c.) and several others.

Since 1937, plants arising from irradiated seeds are always controlled individually. The fertility decrease induced by irradiation has been exactly determined for every X_1-plant and X_1-ear. Therefore the fertility is also known for the plants and ears giving morphological mutations in their offspring. The following survey shows that distinct group-differences exist with regard to X_1-fertility. The eleven mutations of group 3 represent eight or nine different types.

	Group 1	Group 2	Group 3
(A) Average fertility of the mutated X_1-plants	$78{,}5 \pm 6{,}5$ %	$73{,}0 \pm 2{,}7$ %	$51{,}3 \pm 6{,}1$ %
(B) Average fertility of the mutated X_1-ears	$92{,}8 \pm 3{,}9$ %	$74{,}6 \pm 4{,}4$ %	$55{,}8 \pm 7{,}9$ %
Number of cases	4	21	11

D/m $A_{1-2} = 0{,}77$. — $A_{1-3} = 3{,}05$. — $A_{2-3} = 3{,}23$. — $B_{1-2} = 3{,}09$. — $B_{1-3} = 4{,}19$. — $B_{2-3} = 2{,}07$.

Evidently the X_1-sterility increases parallel to the mutation drasticity. The most aberrant mutations (in group 3) differ statistically from the others, both with regard to plant and to ear fertility in the X_1. Similarly, the least striking mutations (in group 1) deviate from the others, at least in ear fertility of the X_1. The analysis of the variance shows that the group differences are real and founded upon specific biological characteristics. The inter/intra-class quotients give P-values between 0,01 and 0,001 for both sets of data. Consequently, in order to appear the drastic mutations require an intense X-ray action on the treated seeds. When crossed to one another erectoides mutations produce what may be called synthetic sterility, indicating that the gene changes causing the erectoides type are accompanied by structural rearrangements. Similarly, the mutations of group 3, originating from extremely sterile X_1-plants and being highly pleiotropic, should combine gene changes with intense genomatic reconstructions.

The more drastic a mutation, the lower is its yield production. Three mutations of group 1, tested by the Swedish Seed Association, give an average yield of 101 (the parent lines = 100), fourteen erectoides mutations a yield of 88, and two drastic mutations a yield of 84. In addition, some drastic mutations are infertile, some are vegetatively poor, some are too late to produce well-developed seed. The *immediate*

evolutionary value of drastic mutations is therefore low. The less drastic erectoides types are rather superior: they imply striking phenotypical changes, show pleiotropy, and attain in some instances yield-figures equal to or higher than the parent lines.

Nevertheless, some drastic mutations are surprisingly good in homozygous condition (mut. »lemma-like glumes»). But they may be further improved by crossing. Of great interest in this connection is SCHIEMANN's description (1930) of a »drastic» mutation in the barley strain »Bethge 3», characterized by short awns and increased straw-stiffness but yielding very poorly. The inferior properties were removed by crossing with »Bethge 2», the straw-stiffness was combined with high production, and, finally, a first-class strain of malt-barley (»Bethge 13») appeared.

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NOTES ON THE COLCHICINE-MEIOSIS OF ALLIUM CERNUUM

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THE influence of colchicine on meiosis in *Allium* was studied by LEVAN in 1939. On that occasion it was concluded that the colchicine treatment caused the complete omission of the despiralization stage between the first and second meiotic division, the division of the centromeres following immediately upon the separation of the bivalents.

As it seemed desirable to check this rather peculiar behaviour on another material, PRAKKEN arranged some experiments during the summers of 1941 and 1942 at the Cyto-genetic Laboratory to study the influence of colchicine and some other c-mitotic substances on the meiosis of rye. Preliminary results thus obtained pointed to there being certain differences in the c-meiotic course of *Secale* and that described in *Allium*. New treatments of meiosis of some *Alliums* were therefore undertaken by the present writers, and some of the old slides left from 1939 were reinvestigated. Unfortunately, other duties have so far made it impossible for us to conclude the study of the rather extensive *Allium* material fixed in 1941—42. The reinvestigation of the old slides, however, has shown without any doubt that in some respects the course of c-meiosis in *Allium cernuum* was misinterpreted by LEVAN. It seems appropriate, therefore, already now to make a short correction on these points.

The renewed study revealed the fact that the interkinetic despiralization stage is not lacking during c-meiosis of *Allium*, although it differs from the normal interkinesis in several respects. It is retarded and rather incomplete. In early prophase of the second division the old (relic) spirals still appear rather regular and tight, so regular and tight, indeed, that these prophase stages were mistaken for telophase despiralization stages.

The main course of the two divisions of colchicine-meiosis in *Allium* is outlined in Fig. 1. It must be emphasized, however, that this picture only represents the main stages as found in the few slides left from the earlier material. The pollen mother cells of these slides had been submitted to colchicine treatment for 24 hours by dipping the

whole inflorescence into a 1 % solution followed by fixing after 48 hours, or a treatment for 48 hours with fixing immediately after the treatment.

Fig. 1 *a* shows the unoriented bivalents of first metaphase—anaphase, the chromosomes of which are just falling apart. The shape of the bivalents in the absence of an active spindle as well as the process of their anaphase separation was described and pictured by LEVAN (l. c., Fig. 2 *a*—*o*). The next picture (Fig. 1 *b*) shows a first anaphase. Now the partner chromosomes of each bivalent are quite free from each

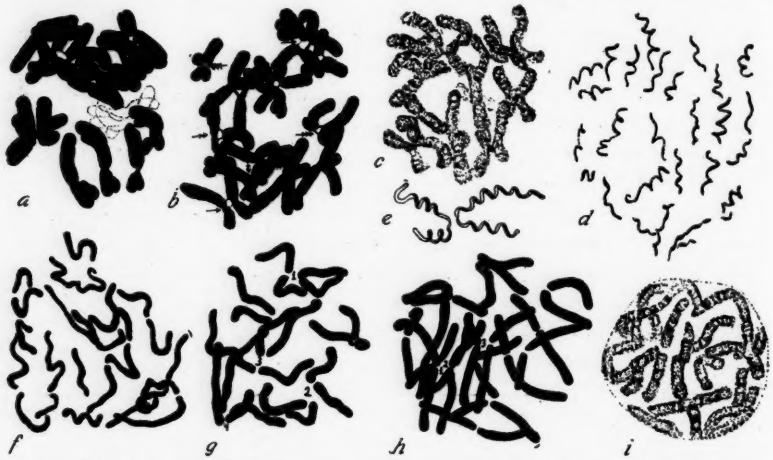

Fig. 1. Colchicine-meiosis in *Allium cernuum* in general outline; *a*: first metaphase—anaphase, the chromosomes just separating; *b*: first anaphase, chromosomes separated; the chromatids of each chromosome are connected by two fine threads (at the arrows of the picture); *c*: first telophase, the chromosomes are weakly stained, swollen and vacuolized; *d*: general appearance of early second prophase, the chromosomes with rather regular and close relic spirals; the elements of the picture represent chromatid arms or parts of arms; *e*: one complete chromosome from the stage of *d*, chromatids probably united at the centromere; *f*: a more advanced second prophase; general appearance, with only part of the arms or chromatids depicted; *g*: still later second prophase; the numbers 1—4 indicate four chromatid pairs, probably still connected at the centromere; *h*: second metaphase—anaphase, chromatids definitively separated; the figures 1—3 indicate three chromatid pairs; only part of the chromatids are depicted; *i*: second telophase, chromosomes similar to those of the first telophase, but somewhat longer and thinner. — × 2000.

other. At first sight even the two sister chromatids of the anaphase chromosomes often seem to be completely separated from each other. A closer inspection, however, reveals that they are still connected at their centromeres. The mutual position of the chromatids indicates that they are

really associated. In many cases also two faintly stained connections between the chromatids were actually seen, one at each side of the centromere. The chromosomes indicated by one-pointed arrows in Fig. 1 *b* consist of horizontally oriented chromatids; in those with two-pointed arrows one chromatid lies horizontally and the other one vertically; in those, finally, with three-pointed arrows both chromatids are vertical, at any rate the portions of the chromatids close to the centromeres. In all these cases two connecting strands could be seen more or less clearly. This may suggest that the connection between the two sister chromatids is not threadlike but rather cylindrical or spherical, the stained threads observed being optical sections of the external layer of the body joining the chromatids. Similar pictures were clearly seen at the corresponding stages of c-meiosis of rye (PRAKKEN, unpublished) and are also characteristic of somatic metaphase chromosomes treated with alkali (LEVAN, unpublished).

First telophase is pictured in Fig. 1 *c*. The chromosomes have the same general structure as in the preceding picture, the chromatids of each chromosome being connected at the centromere. The four arms often show a characteristic cruciform arrangement. At this stage the chromosomes have lost a great deal of their stainability. Their outlines are somewhat rugged, they swell slightly and appear to be vacuolized. No clear spiral structure is visible, although not infrequently there are indications of it. After this stage follows an almost stainless interkinetic stage. The outlines of the chromosomes are now hardly traceable. The chromatic material is gathered within a rounded space, but no clear-cut nuclear membrane was seen.

The stages of the second prophase (Fig. 1 *d—g*) were drawn from another slide. The earliest stages present in this slide were almost completely devoid of staining and represent a continuation of the interkinetic stage just mentioned. This stage passes over into the stage pictured in Fig. 1 *d*, which gives the general appearance of the cells at this stage. The chromosomes, only some of which are depicted, are present as fine, very clear spirals. Owing to a certain fading of the slide it was impossible to reconstruct with certainty whole chromosomes. The various elements of the picture are single chromatid arms or parts of arms. When the slides were new, whole chromosomes were easily traced at this and even a somewhat earlier stage (LEVAN, l. c., Fig. 2 *ff—hh*). Fig. 1 *e* shows one such complete chromosome, reproduced in that paper. The mutual position of the chromosome arms indicates that the centromere of this chromosome is still undivided.

In the two following pictures (Fig. 1 *f* and *g*) the contraction of the chromatids proceeds, at the same time as the originally close and regular relic spiral gradually becomes irregular and finally disappears. In the stages represented by these figures it is very hard to trace the chromatids belonging to the same chromosome. In some cases, however, it was possible to identify such pairs, as, for instance, those indicated by the numbers 1—4 in Fig. 1 *g*. It is quite possible that these chromatids are still connected in pairs by the centromeres, which, anyhow, are now invisible.

The stage represented by Fig. 1 *h* is a second metaphase—anaphase. Still, earlier sister chromatids may be recognized. Some such pairs are marked in the picture by the numbers 1—3. The two chromosomes of each pair are now often lying more or less parallelly beside each other. Usually, the two arms of each chromosome no longer form angles, their shape now often being: — — instead of earlier: \/. This condition indicates that the centromeres are now undoubtedly divided.

Second telophase (Fig. 1 *i*) is very much similar to the first telophase and may easily be confounded with this. The elements of the second telophase, however, are somewhat longer and thinner, as are the chromosomes all through the second division as compared with corresponding stages of the first division. Moreover, it may be clearly seen that during second telophase the chromosomes are not united crosswise. At the second telophase, too, no clear nuclear membrane is seen.

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ABSTRACTS — KURZE MITTEILUNGEN

ARNE MÜNTZING: Different chromosome numbers in root tips and pollen mother cells in a sexual strain of *Poa alpina*.

The apomictic biotypes of *Poa alpina* are characterized by rather high and aneuploid chromosome numbers, the most frequent numbers in Scandinavia being 33 and 38 (MÜNTZING, 1932, 1940). In a sexual strain from Switzerland the chromosome number was found to be oscillating and not lower than 21 (l. c.). In 1942 CHRISTOFF reported that a *Poa alpina* type studied by him was diploid ($2n = 14$) and sexual. The sexuality was demonstrated by embryological investigations. This strain is of unknown origin and was derived from a seed sample obtained from the Botanical Garden of Innsbruck. Thanks to the courtesy of Dr. CHRISTOFF I obtained the remains of the original seed sample from Innsbruck and also seeds of the same strain from plants cultivated in Sofia. Seeds of both these samples were germinated in 1943. The plants were fullgrown in 1944 and also observed in 1945. A total of 40 plants were raised, 20 from »Innsbruck» and 20 from »Sofia». As expected. these two plots show a marked morphological variation as well as differences in viability. This confirms CHRISTOFF's result that the strain is sexual. On an average the plants are more slender than the apomictic *alpina* types and have smaller pollen grains. Otherwise they are typical *Poa alpina* plants. Pollen fertility was found to be variable, but in most plants it was rather good.

In 1944 fixations of p. m. c. were undertaken in several plants in order to study meiosis. New fixations were made in 1945. So far, meiosis has been studied in 5 plants. To my surprise *all these plants were found to have more than 14 chromosomes in the p. m. c.* In *Plant I. 20* a total of 14 metaphases of meiosis were studied, the following configurations being distinguished:

	$9_{II} + 1_I$: 3 cells		$1_{III} + 8_{II}$ 2 cells
	$8_{II} + 3_I$: 3 »		$1_{III} + 7_{II} + 2_I$: 1 cell
	$7_{II} + 5_I$: 1 cell	(Probably)	$2_{III} + 6_{II} + 1_I$: 1 »
(Probably)	$6_{II} + 7_I$: 1 »		

In one cell there were probably $2_{III} + 5_{II} + 2_I$ or $3_{III} + 4_{II} + 2_I$. In another cell, finally, not more than $8_{II} + 2_I$ could be distinguished. In spite of this cell it is quite clear that the typical chromosome number at meiosis in this fixation is *19*.

Fixations of the same plant undertaken in 1945 confirmed that it has more than 14 chromosomes in the p. m. c. So far, only a few first anaphases have been studied. One of them showed quite clearly a 9—9 distribution, another one a 10—8 distribution. Thus, this panicle seems to have 18 chromosomes, one chromosome less than the panicle fixed the previous year.

In *Plant I. 14* a slide from 1944 also showed about 19 chromosomes. In 3 cells there were $9_{II} + 1_I$, in another cell not more than 9_{II} could be distinguished, and in still another one the configuration was 8 or $9_{II} + 3_I$. A slide of the same plant, from a fixation made in 1945, showed diakinesis with more than 7 bivalents. The same result was obtained in *Plant I. 1.*

In *Plant I. 19* preliminary observations of the pollen mitosis were under-

taken. Some meta- and ana-phases clearly showed 9 or 10 chromosomes. In *Plant S. 1*, finally, first anaphases were observed, showing the distributions 9—9 or 8—9.

Root tips of all »Innsbruck»- and »Sofia»-plants available were fixed in May, 1945, and the fixations were repeated later on in the same summer. Though the plants were slowgrowing at this time and it was difficult to get good root tips, chromosome counts could be undertaken in 4 of the 5 plants, in which meiosis had been studied. Without exception these root tips were found to contain 14 chromosomes, the number reported by CHRISTOFF (l. c.). A slight difficulty during these counts is that one chromosome pair has an achromatic constriction which is sometimes very marked. In most cases, however, it is not difficult to see that the two arms of this chromosome really are attached to each other.

Of Plant I. 20 root tips of 3 different fixations, involving 2 clone plants, are available. All the metaphase plates studied show $2n = 14$. Of Plant I. 14 4 different fixations of 2 clone plants gave the same result. The same chromosome number, $2n = 14$, was also observed in I. 1 and S. 1.

More evidence that 14 is the typical chromosome number in the root tips of this strain was obtained by new germinations of the old original seed samples. This resulted in 2 new seedlings of »Innsbruck» and 32 new seedlings of »Sofia». The two Innsbruck seedlings both had $2n = 14$ and the same number was found in 19 of the Sofia plants. 12 Sofia plants were found to be triploid, having either $2n = 21$ or $2n = 21 +$ some extra chromosomes. One plant, finally, had 17 or 18 chromosomes.

From some of the plants first raised a second generation has been obtained, either after open pollination or isolation. Thus, Plant I. 20, showing 19 chromosomes at meiosis, gave one plant with $2n = 14$ after isolation and 9 plants after open pollination. In 7 of these plants the somatic number was again $2n = 14$. In 2 plants most roots had the same number, but a few plates in one root of each plant showed 15 chromosomes. Offspring was also raised after open pollination of Plant I. 1, which had been observed to have more than 14 chromosomes at diakinesis. This offspring consisted of 6 plants, all having $2n = 14$. Two other »Innsbruck» plants behaved in the same way.

The observations described above are sufficient to demonstrate that in this strain the p. m. c. frequently contain a higher chromosome number than the root tips of the same plants. Only one case of the same kind has previously been described in a plant species, viz. the case discovered in *Sorghum* by JANAKI-AMMAL and also studied by DARLINGTON and THOMAS (cf. DARLINGTON and THOMAS, 1941). In *Sorghum* the extra chromosomes are eliminated in the vegetative tissues but are retained in the germ line, i. e. the cells leading to the production of micro- and macro-spores. The extra chromosomes present in the p. m. c. in *Sorghum* are supernumerary fragment chromosomes of the same type as in maize and rye (cf. MÜNTZING, 1946). Some observations indicate a similar situation in the present material of *Poa alpina*. At metaphase of meiosis and also at the pollen mitosis obvious size differences between the chromosomes were observed. The mode of chromosome association at meiosis also strongly indicates a differentiation between a basic genome of 7 chromo-

somes and additional extra chromosomes, capable of forming bivalents and trivalents with each other. It should also be observed that pollen fertility in the plants with extra chromosomes at meiosis was perfectly good.

This peculiar chromosome behaviour is very interesting *per se* but may also shed new light on the chromosomal constitution of the apomictic biotypes of *Poa alpina*. In contrast to the present strain, however, these apomicts have the same chromosome number in root tips and pollen mother cells.

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GÖSTA VON ROSEN: A rapid method for sorting polyploid material.

At a meeting of the Mendelian Society held in the autumn of 1944 Dr. ROBERT LAMM, Alnarp, referred to a notice in Exp. Stat. Record, Vol. 89, No. 3, 1943, p. 285, concerning a simple method of determining chromosome numbers in leaves. The method was published by I. R. MEYER in Stain Technology, Vol. 18, No. 2, 1943, pp. 53—56.

According to MEYER's method new leaves are treated for 1—3 hours with 0,2 per cent colchicine solution, fixed in alcohol — glacial acetic acid — chloroform for 12—24 hours, and stained by the FEULGEN method (see, e. g., DARLINGTON and LA COUR, The handling of chromosomes, p. 110). The preparations can also be preserved in Canada balsam.

With recourse to the division-arresting action of colchicine a simple method has thus been elaborated of obtaining stainable divisions in leaves. MEYER's method, however, can be converted into a really rapid method, and as such ought to constitute a valuable aid in the assortment of polyploid breeding material.

At the Hilleshög Beet Breeding Institute of the Swedish Sugar Company the following procedure is being applied at present for chromosome analysis on leaves. The material consists of beets and peas.

In peas divisions are most easily found in the small leaf-pairs that have not yet grown out of the bracteoles of the stem-shoot, and in beets in the same leaves from early seed stocks.

The leaves are laid direct in about 0,05 per cent colchicine solution, it being seen that the air in the leaves is squeezed out to the greatest possible

extent with a glass rod. The colchicine treatment lasts about two hours and ought not to be prolonged beyond three hours, for after that there is a risk of discoloration and shrinkage of the leaves.

In the case of peas the leaves should be placed direct on the object-glass, the main nerve being dissected out in order to obtain the thinnest possible specimens, and two drops of aceto-carmine made up according to the directions of L. GEITLER (Schnellmethoden der Kern- und Chromosomenuntersuchung, 1940, p. 3) are added. A weak solution stains badly. GEITLER's recipe should preferably be increased by a few drops of a ferric salt solution. The cover-glass is placed loosely over, and the preparation is allowed to boil gently for about half a minute. In serial analysis it is here suitable to use a drying-stand for object-glasses that have been heated with a spirit flame. After being boiled the leaf is soft and easily thinned out by pressure on the cover-glass. An after-staining lasting 10—30 minutes will increase the distinctness of the preparation, but is not necessary. With a good supply of divisions there is no difficulty whatever in finding by this staining method plates suitable for chromosome counting.

The following variant of the method is necessary for the treatment of beets, but may also be used for peas without risk. The colchicine treatment of the beet leaves proceeds in the same way as for peas. The midrib is likewise cut away, but for the staining a mixture of GEITLER's aceto-carmine and chloroform in the proportions 2 : 1 is used. (Note: After shaking.) On heating, a gentle ebullition of the chloroform first appears followed after a short interval by a more vigorous ebullition of water. The total boiling time should be between 1 and 2 minutes. Especially thick leaves require long boiling to admit of being pressed into thin laminae. To avoid formation of the extremely poisonous phosgene gas, that is produced when chloroform is exposed to an open flame, the preparation must in no case be heated over a spirit flame. The most suitable method is to warm the object-glass on a weak electric heating plate.

Following a short after-staining, which is desirable in this case, the preparations are ready for appraisal. In beet preparations the chromosomes do not present themselves so well for counting as in peas, but after some practice diploid and tetraploid plates can be readily distinguished. The total time for treatment of a plant by serial analysis should amount to about 3—5 minutes for a trained cytologist. It may be added that the assessments can be made quite satisfactorily in magnifications up to 1000 ×.

When a sample cannot be judged the same day, it must be transferred from the colchicine solution to Carnoy within 3 hours, in which it can be kept for 24 to 72 hours or more. For long preservation before making determinations the samples are placed in 70 per cent alcohol. The chromosomes in Carnoy-fixed leaves show, however, a tendency to become »waterishly» stained and yield less clear specimens than those stained direct.

Well-defined and clear contrasts in microscopical pictures have been obtained at the Beet Breeding Institute in these serial preparations by using a Zeiss Trichromic Filter.

Hilleshög Beet Breeding Institute, Landskrona, Sweden, June 20th, 1945.